



**AGRICULTURAL RESEARCH INSTITUTE**  
**PUSA**







# PROCEEDINGS

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# PROCEEDINGS OF THE ROYAL SOCIETY.

## SECTION B.—BIOLOGICAL SCIENCES.

### *Reflex Contractions of the Cruralis Muscle in the Decerebrate and Spinal Frog.*

By N. B. LAUGHTON, B.A.

(Communicated by Sir Charles Sherrington, P.R.S. Received December 15, 1922.)

(From the Department of Physiology, Western University Medical School,  
London, Canada.)

The object of this research is to compare the reflex contractions of the cruralis muscle in the decerebrate and spinal frog in response to a single break induction shock, applied to the ipsilateral sciatic nerve.

Sherrington (5) studied the limb reflexes in the decerebrate and spinal cat. He showed that in the decerebrate preparation the reflex contraction of an extensor muscle was accompanied by an "autogenous tonic reflex" which prolonged the contraction. This "autogenous tonic reflex" was absent from the reflex contractions of the flexors in the decerebrate, and from the extensors in the decapitate, preparations. Sherrington (5) and Sherrington and Sowton (6) showed that in the spinal cat the reflex contraction of the tibialis anticus muscle was higher and had a more rapid ascent than in the decerebrate cat. Sherrington concluded that the mid-brain exercises an inhibitory influence over the flexor limb centres in the cord. Head (2) arrived at similar conclusions in stating that, on removal of the dominant physiological centres of the central nervous system, the lower centres in the cord are released from control.

In the hind limb of the decerebrate frog, the reflex tonus is in the flexor muscles, a condition unlike that in the decerebrate cat and dog where the extensor muscles are in exaggerated tonic contraction. Sherrington (4) pointed out that the postural tone in spinal frogs is marked in the flexors and very low in the extensors. Since no previous work was found dealing with the reflex



contraction of the cruralis muscle of the frog on stimulation of the ipsilateral sciatic nerve, the present investigation was undertaken.

### *Methods.*

The stimulus used in these experiments was a single break induction shock from a "Kershaw" coil. The shock was obtained by breaking the primary circuit by a key, which was opened by the swing of a pendulum, released by a catch. A current of 0.8 ampères supplied from a storage cell flowed in the primary circuit in all the experiments. The threshold stimulus for the sciatic nerve was obtained with the secondary beyond 250 mm., but in order to obtain a maximal contraction the secondary was placed at 150 mm. The stimulus was applied by means of electrodes after Sherrington's pattern, the kathode for break shocks being proximal.

The frogs employed in this work were decerebrated from ten days to two weeks prior to the experiments. The tendon of the triceps muscle was isolated and in consideration of Lombard's (3) work, in which he showed that the tensor fasciæ latæ and glutæus magnus, two components of the triceps, are strong flexors of the thigh, these muscles were cut at their origins. The cruralis muscle, an extensor of the leg, was left, with the nerve supply intact for recording. The remaining muscles of the thigh were immobilized by cutting their nerves or by tenotomy. The leg was secured in a suitable clamp so that occasional movements of the frog could not influence the myogram. The tendon of the triceps was connected to an isometric myograph by means of a thread. Records were taken on a rapidly moving drum, in response to a break shock applied to the ipsilateral sciatic nerve.

The spinal preparations were obtained from the decerebrate preparations either by pithing the mid-brain or by sectioning the cord at the level of the second vertebra. In some of the experiments I recorded reflex contractions during the period of spinal shock, and found that the reflex contractions reached their maximum in about fifteen minutes after the animal had been made spinal. In the remainder of the experiments, accordingly, I waited until such an interval had elapsed before recording the contractions.

Du Bois Reymond (1) showed that if either the tibial or peroneal nerve in the frog is stimulated, after section of the sciatic above the point of branching, a "Paradoxical Contraction" occurs in the muscle supplied by the other nerve. In order to exclude this fallacy, the cord was destroyed at the termination of the experiment, and it was found that no contractions of any kind occurred in the cruralis muscle, on stimulating the ipsilateral sciatic nerve.

*Results.*

The decerebrate frogs assumed the characteristic posture. The head was slightly elevated, the hind legs flexed and the forelegs extended; when jumping the animals avoided objects. On stroking the sides, the croak reflex and the increased tonus in the extensor muscles as described by Verworn (7) were readily elicited.

In the decerebrate frog the reflex contraction of the cruralis muscle on stimulation of the ipsilateral sciatic nerve showed very characteristic features.

FIG. 1.—Reflex contraction of the cruralis muscle of the decerebrate frog in response to a single break shock applied to the ipsilateral sciatic nerve. Sec. coil at 150 mm.

The ascent of the myogram was gradual. The descent at first was abrupt, this phase being followed by a tonic contraction of the muscle which pro-

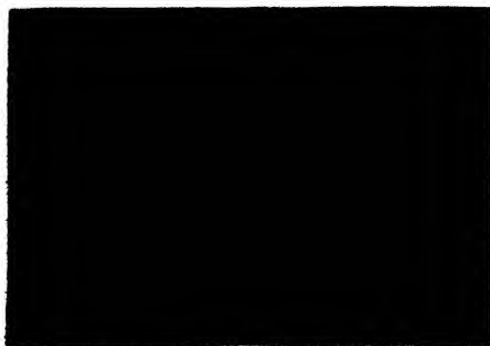


FIG. 2.—Reflex contraction of the cruralis muscle of the spinal frog in response to a single break shock applied to the ipsilateral sciatic nerve. Sec. coil at 150 mm. Figs. 1 and 2 from the same animal.

longed the relaxation period. In the spinal preparation the myogram showed different characteristics.

The ascent was more abrupt than in the decerebrate animal. This was followed by a rapid descent which reached the base line in the majority of cases. There was no tonic effect as in the myograms obtained from the decerebrate preparations.

It is further found that the latent period and the time for reaching the maximal height were greater in the decerebrate than in the spinal frog. For example, in the tracings shown in figs. 1 and 2 the latent period in the decerebrate frog was 0.03 sec., and the time to reach the maximum height was 0.2 sec. In the spinal frog, the latent period was 0.015 sec., and the time to reach the maximum height was 0.14 sec. The relative heights obtained in the decerebrate and spinal frogs were not constant. In fifty per cent. of the experiments the height of contraction was higher in the decerebrate than in the spinal preparation. In the remaining fifty per cent. the height of the contraction in the spinal was greater than that in the decerebrate frog.

#### *Discussion.*

The reflex tonic contraction obtained in the decerebrate frog, from the cruralis muscle, on stimulating the ipsilateral sciatic nerve is probably similar to the "autogenous tonic reflex" described by Sherrington (5) in the extensor muscles of the decerebrate cat. In the spinal cat and spinal frog no such effects are obtained. The reflex contraction of the cruralis muscle, in fifty per cent. of the frogs studied, resembles that of the flexor muscles in the spinal cat, in that the rate of ascent and contraction heights are greater than in the same animals in the decerebrate condition. The remaining fifty per cent. of the frogs differed from the above, in that the contraction height was lower in the spinal condition than in the decerebrate. In all the spinal frogs the latent period was shorter than in the decerebrate condition.

The more rapid increment of height, the shorter latency, and the increased height in fifty per cent. of the spinal frogs, indicates that the mid-brain exercises an inhibitory influence over the extensor centres in the spinal cord of the frog. This inhibitory influence is probably similar to the inhibitory influence of the mid-brain over the flexor centres in the spinal cord of the cat, as pointed out by Sherrington (6). I am unable to explain why the contraction height in the spinal frogs is less than that in the decerebrate in fifty per cent. of the experiments. Regarding this question further experiments are in progress.

*Summary.*

1. In the decerebrate frog there was a prolonged tonic after-effect in the contraction of the cruralis muscle on reflex stimulation of the ipsilateral sciatic nerve. No such tonic effect was observed in the cruralis muscle of the spinal preparation on stimulation of the same nerve.

2. A shorter latent period and a more rapid increment of height were marked in the spinal preparations.

3. During spinal shock the height of the reflex contraction in the spinal frog is not maximal.

4. In fifty per cent. of the experiments, the height of the myogram was greater in the decerebrate than in the spinal preparations. In the other fifty per cent. of the experiments, the height of the contractions was greater in the spinal than in the decerebrate frogs.

This work was undertaken at the suggestion of Prof. F. R. Miller, and I wish to take this opportunity of expressing my thanks for his kind criticism and assistance.

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- (4) Sherrington, 'Brain,' vol. 38, p. 191 (1915).
- (5) Sherrington, 'Jour. Physiol.,' vol. 40, p. 28 (1910).
- (6) Sherrington and Sowton, 'Jour. Physiol.,' vol. 49, p. 331 (1914-15).
- (7) Verworn, 'Allgemeine Physiologie,' p. 384, Vierte Aufl. (1903).

### *The Mechanism of Ciliary Movement. III.—The Effect of Temperature.*

By J. GRAY, M.A., Fellow of King's College, Cambridge.

(Communicated by Prof. J. S. Gardiner, F.R.S. Received February 10, 1923.)

The general effect of temperature on the rate of ciliary movement has been known for many years. In 1858, Calliburcés(1) determined the rate of movement of the cilia on the frog's œsophagus, by observing the speed of rotation of a small glass cylinder laid in contact with the ciliated surface; he found that an increase in temperature caused a marked increase in the speed of rotation. Similar results, obtained by various methods, were recorded by Roth(11), Engelmann(2), and Rossbach(10), but in no case were the observations sufficient for quantitative analysis.

Owing to the fact that ciliated surfaces are liable to be contaminated by strands of mucus, data based on the speed imparted by the cilia to a revolving drum are liable to considerable experimental error; this error is also increased by the mechanical friction involved by such instruments. The only satisfactory method of estimating the speed of the cilia on the gills of *Mytilus* appears to be by a determination of the rate of transportation of fine particles over the surface of the tissue; such particles can be watched under the low power of the microscope, and any irregularities due to the presence of mucus can be detected. The method adopted in the following experiments was to determine the time required to move at a uniform rate a very small circular plate of platinum over a distance of 1 cm. The tissue was fixed by glass weights to the bottom of a small glass dish, across the base of which were marked two lines 1 cm. apart. The relative speed at different temperatures was recorded, using the speed at 15° as an arbitrary unit of 100.

In order to regulate the temperature of the tissue, two methods have been used:—

(a) The tissue was held in position at the bottom of a small beaker of sea-water, which stood inside a water-jacket. The platinum weight was then dropped on to the tissue and its rate measured over the standard centimetre. By adjusting the temperature of the water in the jacket it was found quite simple to keep the temperature of the beaker itself constant to  $\frac{1}{2}^{\circ}$  C. for a period long enough in which to make five determinations of the speed of the cilia.

(b) A small microscope stage was made in which the temperature was

controlled by an electrical resistance. By varying the current, different temperatures could be obtained. The actual temperature of the drop was determined by a small thermocouple placed in contact with the tissue.

If the ciliated tissue be suddenly plunged into water at different temperatures the direct effect is as follows :—

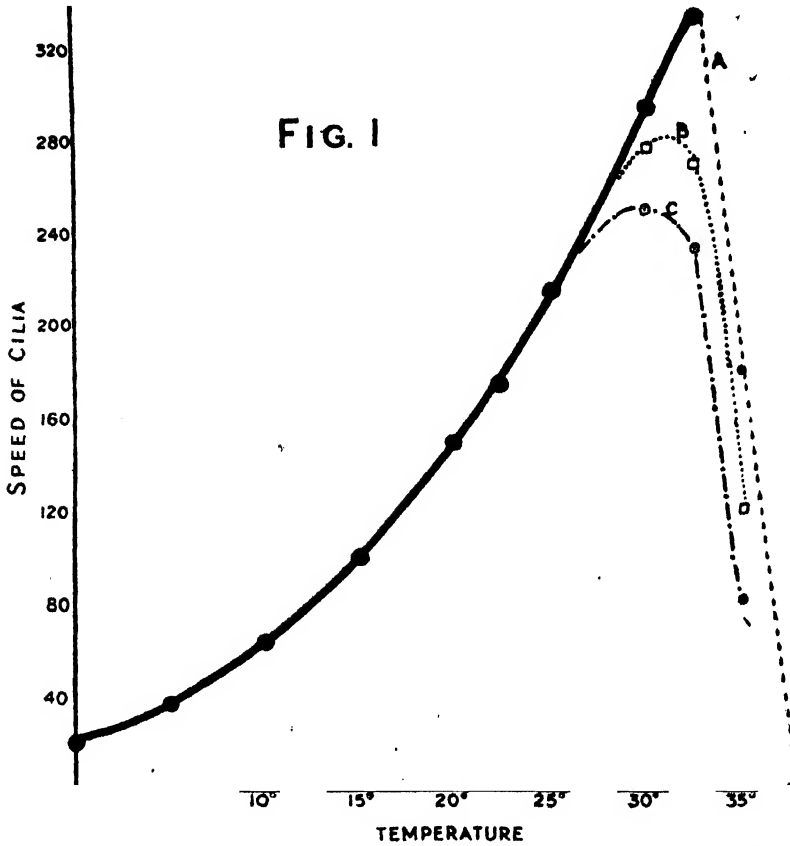
Table I.

Temperature.	Effect on cilia.
0-32.5	Progressive increase of speed with increasing temperature. Amplitude of beat normal.
34	Very rapid beat. Amplitude reduced.
36	Very rapid beat. Amplitude very small, causing a rapid flickering movement.
37.5-38.5	Speed of beat rapidly reduced.
40	Cilia stationary in relaxed position.
45	Cilia pass into the contracted position.
47	Cilia opaque. Irreversibly injured.

From 0°-36° the direct effect of temperature on the movement of the cilia is completely and instantaneously reversible. For example, the effect of 36° on the amplitude of the stroke is completely lost as soon as the temperature falls to about 33°. The effect of temperatures at which the speed of the beat begins to fall (viz., 37.5°-40°) is also reversible, but the recovery is not instantaneous on lowering the temperature. A brief exposure to 40° may require 20 minutes at 15°, in order that the recovery of any of the cilia may be complete.

In addition to the direct effect upon the ciliary mechanism, high temperatures have a profound effect on the cell. If the temperature of the tissue be varied from 0°-28°, even prolonged experiments involve no loss of vigour. Above this temperature, however, the tissue begins, sooner or later, to show signs of unhealthiness; individual cells detach themselves from the epithelium, and in some cases the cilia themselves are destroyed. The higher the temperature the more rapidly does this process become evident, and the greater is the number of cells affected. Tissues from different animals have different degrees of susceptibility. Whereas the "disintegrative" effect of high temperatures on the tissue varies with the time of exposure, it should be mentioned that the direct effect of such temperatures on the ciliary mechanism does not vary with the time of exposure, since in this case each temperature has its characteristic effect (see Table I) which does not alter with prolonged exposure. Some of these facts are illustrated quantitatively in Table II and Curve A, fig. 1. This curve is constructed

from a large series of observations, all of which showed remarkable agreement with each other. The conditions under which the data for this



curve were obtained were such that the speed was determined within 2 to 4 minutes of reaching the experimental temperature.

Table II.

Temperature.	Speed in mm. per sec.	Relative speed. Speed at 15° = 100.	Temperature interval.	$Q_{10}$ .
°			°	
0	0.08	20	0-10	3.1
5	0.15	38	5-15	2.7
10	0.26	64	10-20	2.3
15	0.40	100	15-25	2.15
20	0.60	160	20-30	1.95
22.5	0.70	174	22.5-32.5	1.92
25	0.86	215		
30	1.17	294		
32.5	1.33	334		

It is clear that there is a gradual fall in the temperature coefficient with increasing temperature. Between  $0^{\circ}$  and  $30^{\circ}$  the process is entirely reversible, showing that the fall in the value of  $Q_{10}$  is not due to any irreversible injury. Above  $33^{\circ}$ , however, the speed of the particle falls off very rapidly with increasing temperature, and at  $37.5^{\circ}$  the cilia cease to create any detectable current. This rapid loss of efficiency coincides with the rapid reduction in the amplitude of the ciliary beat.

If the tissue be exposed to temperatures above  $33^{\circ}$ , it is invariably found that, on lowering the temperature, the activity of the cilia never reaches its original value. This phenomenon varies with the following factors: (a) the physiological condition of the tissue; (b) the maximum temperature to which the tissue is exposed; (c) the time of exposure to the high temperature. If the experimental temperature be fairly high (for example,  $35^{\circ}$ ) the effect is very well marked, and can be seen to be due to the disintegration of the ciliated epithelium described above.

The effect of this process on the mechanical activity of the tissue is illustrated by Curves B and C in fig. 1, which show the rate of beat after exposures of  $\frac{1}{2}$  hour and 1 hour respectively.

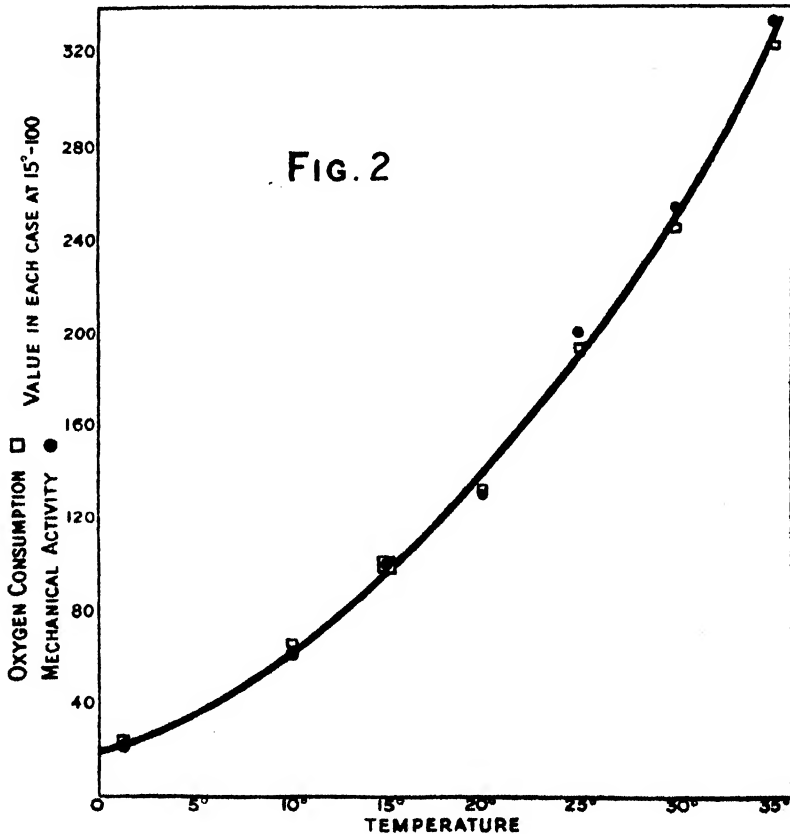
Eliminating the disintegrative effect of high temperatures on the stability of the cell, it is clear from Table I that from  $33^{\circ}$  upwards the activity of the ciliary mechanism is directly affected by temperature. As far as can be determined by microscopic observation, the reduction in the amplitude of the beat is completely and instantaneously removed if the temperature is lowered to  $30^{\circ}$  or less. At  $38^{\circ}$ – $39^{\circ}$ , however, there is a distinct reduction in the speed of the beat, so that at  $40^{\circ}$  all movement has ceased; if the temperature be now reduced to about  $15^{\circ}$ , recovery does not occur at once. Some cells are completely destroyed (disintegrative action), but the remainder eventually recover. The recovery begins by a slow beat of normal amplitude, the rate of beat gradually increases, until finally it appears to reach its normal value.

Two suggestions have been advanced as explanations of the depressant effect of high temperatures upon biological processes: (i) that at high temperatures the cells suffer from a lack of oxygen, in other words, one or more of the activities of the cell which are dependent upon oxygen go on quicker than they can be supplied with oxygen, and the tissue becomes fatigued; (ii) at high temperatures one or more of the endocellular enzymes cease to be stable, and are gradually destroyed.

Since ciliary activity depends upon the presence of oxygen, it seemed desirable to determine the relationship between the mechanical activity of the cell and the amount of oxygen consumed.



All the experiments were performed with Barcroft manometers. It was found essential, however, to modify the form of the respiration chamber in such a way as to ensure complete ventilation of all the tissue used. Unless such precautions are taken, the value of the temperature coefficient is greatly reduced at high temperatures. The type of apparatus eventually adopted was that in use in the Biochemical Laboratory, Cambridge. With this form only three gill lamellæ were used in each experiment, and the conditions were as nearly as possible those under which the mechanical activity of the tissue was measured. The results (Table III and fig. 2) show



clearly that the oxygen consumption is almost exactly proportional to the mechanical activity.

Until, therefore, the temperature is high enough to interfere with either the amplitude of the ciliary stroke, or with the stability of the cells, the degree of mechanical activity is exactly paralleled by the amount of oxygen absorbed. There is thus definite evidence against the view that, as the

Table III.

Temperature.	Mechanical activity.	Oxygen consumption of tissue in bulk.	Temperature interval.	Q <sub>10</sub> -Mechanical activity.	Q <sub>10</sub> -Oxygen consumption.
°			°		
1	24	26	1-10	3.1	3.0
10	60	65	5-15	2.7	2.6
15	100	100	10-20	2.3	2.17
20	180	180	15-25	2.15	2.0
15	100	100	20-30	1.95	1.91
20	132	133	22.5-32.5	1.92	1.90
25	185	184			
30	253	246			
15	100	100			
25	215	203			
32.5	334	324			

temperature is increased, there is a gradual lack of ability to maintain the requisite level of oxidation. Further, if the temperature is raised from 10° to 25°, and maintained at the higher level for as much as 2 hours, on reducing the temperature to 10° the rate of oxygen consumption returns to its original level at once. If, however, the ventilation of the tissue is not complete, then the oxygen consumption, and probably also the mechanical activity, shows an abnormally low value at high temperatures.

These results agree with those of Henze (5) who found that the effect of temperature on the respiration of sea-anemones had a much lower temperature coefficient than has that of well aerated animals or tissues, and that the respiration of the former depended on the amount of available oxygen.

With increasing temperature the concentration of oxygen in sea-water falls very distinctly. It may be pointed out that if it be assumed that the rate of oxygen consumption be dependent on the concentration of oxygen in the medium then the temperature coefficient from 10°-30° remains quite constant throughout this wide range at 2.40 (Table IV).

Table IV.

Temperature.	O <sub>2</sub> concentration in c.c. per litre sea-water.	O <sub>2</sub> consumed.	O <sub>2</sub> consumed corrected for concentration.	Q <sub>10</sub> .
°				
10	6.4	64	80	
20	5.85	150	224	2.40
30	4.5	294	535	2.39

It may or may not be a simple coincidence that this value agrees very closely with that determined by Hill (6) for the rate of development of an isometric muscular twitch.

Since the temperature coefficients of oxygen consumption and of mechanical activity are of the same value, and since mechanical activity depends upon the presence of oxygen, it must be concluded that either (1) the rate of oxygen consumption determines the degree of mechanical activity, or (2) the rate of mechanical activity determines the rate of oxygen consumption. In the latter case, however, it is necessary to suppose that unless all the oxygen consumed is concerned with the products of mechanical activity, then the "residual" oxidation of the cell, and the oxidation directly concerned with mechanical activity, are of the same nature and have the same temperature coefficient.

It is obvious that there is definite evidence against the view that a rise in temperature involves a lack of oxygen in a well ventilated tissue. It is possible, however, that the mechanical activity is controlled at high temperatures by the amount of available oxygen.

From a study of the effect of high temperatures upon the rate of assimilation of green leaves it has been suggested that the critical effect of temperature is due to a destruction of endocellular enzymes, since the effect of temperature on the activity of living organisms is paralleled by its effect on the activity of enzymes *in vitro*. The effect of temperature on the rate of oxidation of ciliated tissue is exactly parallel to its effect upon the assimilation of green leaves (Matthaei, (9)). At high temperatures the rate of oxidation falls off with increased time of exposure. As with green leaves the following factors are involved—(1) the physiological condition of the tissue, (2) the temperature used in the experiment, (3) the duration of the experiment. In the case of the animal tissue, it is clear that these are the factors involved in the "disintegrative" action of temperature on the epithelium, whilst these factors are not involved in the direct effect of temperature on the mechanical mechanism.

#### Experiment I.

Temperature 32.5°.

1st 30'	of experiment	oxygen absorbed	was	154.
2nd 30'	"	"	"	156.
3rd 30'	"	"	"	136.
4th 30'	"	"	"	95.
6th 30'	"	"	"	88.

#### Experiment II.

Temperature 36°.

1st 30'	oxygen consumption	was	74.
2nd 30'	"	"	60.
3rd 30'	"	"	31.

The ability of any particular specimen of tissue to maintain its full rate of oxidation at high temperatures is always reflected in its microscopic appearance after the experiment. Tissues which had been exposed to high temperature and in which the oxygen consumption had fallen very considerably, invariably show signs of much disintegrative action; when the fall in oxidation was small, the amount of disintegration was also small. It may be concluded, therefore, that the falling-off in the oxidation is to be correlated with the secondary effect of temperature on the tissue, and not with the direct effect on the mechanical mechanism.

If the direct effect of the temperature on the activity is to be associated with the destruction of an enzyme, then it would seem as though this enzyme must be hydrolytic in nature and not oxidative. The cell contains both catalase and peroxidase, but the activity of the tissue to liberate oxygen from hydrogen peroxide, and to form indophenol blue, does not appear to be materially affected by heating to  $45^{\circ}$ ; which is consistent with the fact that the point of thermal instability of these enzymes is in the neighbourhood of  $60^{\circ}$ . On the other hand the thermal stability of hydrolytic enzymes is much lower. The destruction of some such hydrolytic enzyme would explain why the recovery of the beat at low temperature does not take place at once.

At present it is only possible to speculate on the probable cause of the reduction of the amplitude of the beat between  $34^{\circ}$  and  $38^{\circ}$ . It is possible that at this temperature the rate at which the process of contraction is induced in the cilium is more rapid than the process of relaxation, so that relaxation begins before contraction is complete. Such a condition would be reversible as soon as the temperature was lowered. The complete state of contraction which occurs at  $45^{\circ}$  may possibly be due to the direct effect of the temperature upon the fibres of the cilium. The contraction which can be induced in a fibre of catgut was demonstrated by Engelmann (3), who regarded it as comparable to the normal contraction of a muscle. It is here suggested as equivalent to the prolonged contraction produced in a cilium at  $45^{\circ}$  C. All attempts to isolate or detect an endocellular substance which coagulates at this temperature have failed, and since the effect of  $45^{\circ}$  C. on the cilium is reversible, it can hardly be due to any process of irreversible heat coagulation.

It remains to consider how far the effect of temperature upon the activity of cilia is paralleled by its effect on other forms of contractile protoplasm. Apart from a similarity in the contraction effect at high temperatures, and, to a certain extent, the nature of the temperature coefficient, no data are available for comparison with amoeboid or streaming movement. Up to a certain point, a comparison may be made between automatic cilia and skeletal muscle if the latter be subjected to a rhythmical series of shocks of maximal intensity.

The effect of temperature on such a muscle preparation has been observed by Howell (7). Between about 17° C. and 30° C. the height of an isotonic contraction rises with increasing temperature. At about 32°, however, the height of the contraction falls very rapidly until at 36° only a very slight response occurs; at 38° the muscle passes into the highly contracted condition characteristic of heat rigor. The closest parallel to automatic cilia lies, however, in cardiac muscle, especially the automatic region of the sinus and the auricles. In the case of the frog, the speed of the beat rapidly increases, with rising temperature, up to 30°. As with cilia, the temperature coefficient varies with the scale of temperature at which it is measured; over the interval 30.5°–1.2° the coefficient varies from 3.1 to 1.3 (Snyder (12)). Beyond 30° the amplitude of the beat falls markedly and eventually gives rise to a fibrillar condition just prior to the point at which the heart comes to rest. Parallel also to the behaviour of ciliated epithelium, the oxygen consumption of the heart is directly proportional to the speed of the beat (Evans (4)). The only detectable difference in the relation of cilia and of any cardiac muscle to changes in temperature is the fact that in the case of the mammalian heart the speed of the beat and the oxygen consumption appear to be a linear function of the temperature (Knowlton and Starling (8)).

It is, therefore, possible to conceive that the motile mechanisms of ciliated cells and of cardiac muscle may be found to have much in common.

#### *Summary.*

1. Between 0° and 33° C. the speed of the cilia on the gills of *Mytilus* increases with a rise in temperature, although the amplitude remains normal. Between 34°–40° there is a marked falling-off in the amplitude of the beat, followed by a reduction in speed. At 40° the cilia came to rest in the relaxed position. At 45° the cilia occupy the contracted position.
2. The temperature coefficient of movement between 0° and 32.5° varies from 3.1–1.92.
3. High temperatures produce a destructive effect on individual cells of the epithelium.
4. In well aerated tissue the oxygen consumption is directly proportional to the speed of the beat between 0°–30° C.
5. At temperatures above 30° the initial oxygen consumption is not maintained. This reduction is due to the disintegrative effect of the temperature on the epithelium.
6. The effect of temperature on the activity of cilia is closely parallel to its effect on other types of contractile protoplasm.

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*The Pigmentary Effector System. III.—Colour Response in the Hypophysectomised Frog.\**

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[PLATE I.]

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§ 1. *Introduction.*

The synchronous character of colour response in all parts of the body among the Amphibia has prompted a large body of investigators to seek for the existence of some co-ordinating mechanism underlying the normal

\* The expenses of this research were defrayed by a grant from the Earl of Moray Fund.

reaction of the chromatophores. Nevertheless, no satisfactory account of such a mechanism has yet been put forward. The failure of earlier efforts is chiefly explicable for two reasons. Partly because, when the physiology of pigmentary changes first seriously excited interest, in the fifties and sixties of the last century, attention had not as yet become directed by the discoveries of Schafer and others to the alternative of endocrine factors in controlling colour response independently of the nervous system. And though the action of adrenalin in promoting melanophore contraction had later suggested to several Continental workers such an interpretation, it has only become possible during the past two years, through the work of the present writers on post-pituitary\* extracts, to identify a second autacoid system tending to promote the opposite phase of melanophore expansion. That the minds of earlier investigators were unduly hampered by the desire to interpret the control of pigmentary response through nervous agencies must be regarded as the principal explanation of the variable results recorded by numerous and equally competent and reliable witnesses with regard to such straightforward issues as the effects of nerve section and stimulation upon the condition of the chromatophores.

It is now proposed to complete the attempt to provide a coherent account of the pigmentary changes of the common frog by supplementing our previous studies on the pituitary melanophore reaction with a comparison between colour response in the normal and hypophysectomised frog.

## § 2. *The Technique of Hypophysectomy.*

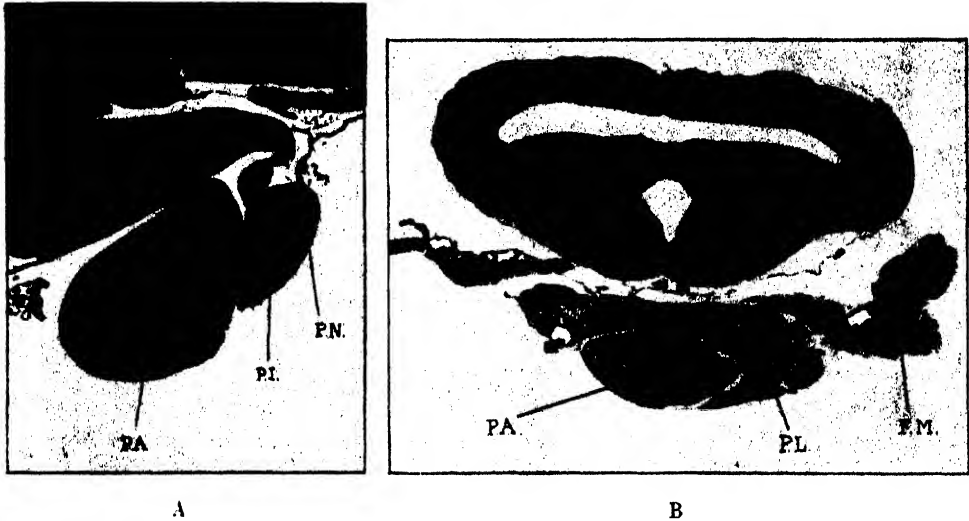
A method for the removal of the pituitary in the frog has already been described by one of the authors. References to previous attempts are recorded in that account (Hogben, 1923).

When approached from below, the pars anterior of the frog is very prominent, owing to its pink hue and large size. The chief difficulty lies in penetrating the base of the skull, the localisation of the organ presenting no difficulty, owing to its characteristic orientation with respect to the dagger-like parasphenoid and the possibility in young animals of actually seeing where it lies through the semi-translucent bone. Once a suitable aperture has been made, the hypophysis, or either of its constituent parts, can be readily removed without the least laceration, seeing that neither pars anterior, intermedia, nor tuberalis are histologically continuous with one

\* We have employed the term "post-pituitary" to designate extracts of the posterior lobe of the pituitary gland, to avoid a periphrasis in the confused state of existing nomenclature for pituitary preparations.

another nor with the floor of the brain, and may be separated by gentle pressure or stress with cataract needle or seeker. (Text-fig. 1.)

After deep ether anaesthesia, the frog is laid on its back on a sloping rest,



TEXT-FIG. 1.

- A. Longitudinal section.—Pituitary gland of the frog (*Rana temporaria*); *P.A.*, pars anterior; *P.I.*, pars intermedia; *P.N.*, pars nervosa.  
 B. Obliquely transverse section of the brain and pituitary, showing the lateral extension of the posterior lobe (*P.L.*, pars intermedia and nervosa) and the fatty masses referred to in the text.

the mouth being opened to the fullest extent possible with a forceps having the extremities bent outwards, and applied to the anterior mid-point of the upper and lower jaws. A triangular flap of the mucous membrane of the roof of the mouth is deflected backwards to expose the parasphenoid, which may be washed with a dilute solution of some innocuous antiseptic. The rose burr of a dental drill is now applied to the mid-point of the parasphenoid in a transverse line defined by the anterior margin of the lateral processes of the bone. The bone is worn down until a thin lamina of cartilage alone covers the pituitary, now readily visible under the binocular microscope. With the aid of the latter the operation is completed. An aperture large enough to expose the anterior lobe, the greyish posterior lobe lying beneath it, and the fatty masses which flank them is opened with a cataract needle by cutting through the thin lamina of cartilage overlying the pituitary. A flap of cartilage is left, so that the latter may be reinstated after the operation. Gentle pressure is then sufficient to detach the anterior lobe, if this only is to be removed. For extirpation of the



entire gland a different procedure is adopted. A glass pipette, with aperture just wide enough to admit the gland, and connected by a suitable length of rubber tubing, with a filter pump kept in continuous action throughout the operation, is held in readiness, while the connexions of the fatty masses are severed by a cataract needle rotated in the opening through the roof of the mouth. The posterior lobe now lies loosely in this cavity, which is swabbed out with cotton-wool. The glass pipette is then applied to the aperture and the gland instantaneously sucked up, leaving behind a clear view of the brain free of blood or any remnants of the hypophysis. The use of the dental machine we owe to the suggestion of our friend and colleague, Dr. F. A. E. Crew, Director of the Animal Breeding Research Department, in whose laboratory the research was carried out. The same method of penetration applied below the optic chiasma was employed for section of the optic nerve.

### § 3. *Normal Pigmentary Responses of the Frog.*

It is imperative, at this stage, to insert a definite statement of the pigmentary changes which occur in the common frog in response to such normal environic stimuli as light, temperature, and humidity. Extensive experiments were initiated last summer, and have been checked by continuous observation during the past 2 years, during which period more than 2,000 frogs have been employed in the study of different aspects of colour response by the present writers. Large numbers of frogs have been kept in light and darkness, white and black background, moist and dry condition, high and low temperature. It is sufficient here to summarise the results:—

Table I.—Normal Pigment Responses of the Common Frog.

	20° C.	10° C.
Light background—		
(a) Dry	Pallor	Generally pale
(b) Moist	Pallor (Epidermal melanophores expanded)	Darkening
Shade or dark background—		
(a) Dry .....	Pallor	Partial darkening (Epidermal melanophores contract)
(b) Moist	Darkening	Darkening
Darkness—		
(a) Dry	Pallor	Partial darkening
(b) Moist	Darkening	Darkening

From this Table it will be seen that moisture, cold, and shade, promote melanophore response of the same type as post-pituitary administration. Generally, in warm, well illuminated situations, the epidermal melanophores expand in the presence of sufficient moisture, while in dry weakly illuminated situation, at low temperature, the dermal melanophores are expanded, but the epidermal commonly contracted, *i.e.*, the dermal melanophores are more sensitive to cold, and the epidermal more sensitive to moisture. These responses are always slow and accumulative in their intensity. The production of pallor is not accomplished in less than half-an-hour at warm temperature, and may extend over a day in colder condition. Darkening of the skin often takes several hours at high temperature (above 20° C.), and never takes less than three-quarters of an hour. Frogs placed in cool, shady, and moist surroundings, are often noticeably darker after 24 hours, as compared with their condition 12 hours after the beginning of the treatment. We attach no little importance to the prolonged latent period and accumulative nature of the response for the interpretation of the co-ordinating processes in normal colour responses. In view of experiments to be described later, it may be well to repeat explicitly, in this connection, that low temperatures favour expansion, and high temperatures promote contraction, of the melanophores.

#### § 4. *Action of Pituitary Extract on the Isolated Limb.*

The effects of adrenaline and of various drugs upon pigment response in the intact frog, as recorded in the preceding contribution of this series, do not necessarily point to a nervous control of the melanophores. It is possible that their action is indirect, through the vaso-motor changes evoked, rather than a local influence directly exercised upon the melanophores themselves. In the case of the pituitary autacoid this is not so, for two reasons: (a) The action of pituitary extracts upon the isolated skin; (b) the evidence adduced elsewhere that the melanophore stimulant is not identical with the pressor component of pituitary extracts. This direct character of the pituitary reaction upon melanophores is of so much importance to our present thesis, that we here insert an additional experiment which may prove suitable for demonstrating the physiological activity of pituitary extracts to students in laboratory class work.

A *pale* frog is decapitated instantaneously, and the limbs are immediately ligatured above the knee-joint. After the limbs have been severed and separately placed in watch-glasses containing Ringer's solution, 0.2 c.c. of saline is injected into the lymph sac of one, and 0.2 of a 0.1 per cent. saline extract of posterior lobe into that of the other. By means of cotton, if necessary, the

web is extended in each watch-glass, which is placed on the stage of a student's microscope for examination with the low power. After 20 minutes to  $\frac{1}{2}$  hour the melanophores of the second leg should be fully expanded, while those of the first should be completely contracted. We have carried out this experiment repeatedly at  $22^{\circ}\text{C}.$ , but at lower temperature the response may require a longer period of time.

### § 5. *The Effects of Pituitary Removal.*

*First Series.*—Five dark frogs, Nos. 4, 62, 27, 26, 12, were deprived of both lobes; 6 hours after the operation pallor ensued. They were then placed in glass containers with a little water in a cool room ( $10^{\circ}\text{C}.$ ) on a dark background. Five pale frogs were placed in a container with water alongside of the experimental animals in a similar situation. These underwent darkening within 4 hours and remained dark continuously. The webs of both sets of frogs were examined daily with the microscope. In the hypophysectomised frogs the epidermal and dermal melanophores remained in a condition of maximum contraction. In these animals the xantholeucophores, on the other hand, displayed a reticulate appearance, being more remarkably expanded than in animals made pale by administration of adrenaline. In the controls the melanophores remained in the reticulate condition, and the xantholeucophores were contracted to a spherical contour. No. 4 of the experimental animals was killed a week later for microscopic preparation of the skin. No. 27 died a fortnight after the operation. Nos. 26 and 62 were killed for microscopic purposes 17 days after the operation. No. 12 lived for a month.

In every case the experimental animals remained pale, with complete contraction of the melanophores and reticulate expansion of the xantholeucophores, till they died or were killed.

*Second Series.*—In a second series of experiments it was decided to make the controls as rigid as possible by recording the effects of: (1) Removal of the anterior lobe alone; (2) exposure of the brain without damage to the pituitary; (3) exposure of the brain followed by section of the optic nerves at the chiasma.

Five pale animals, Nos. 36, 73, 37, 76, 38, were deprived of the anterior lobe of the pituitary alone. Three hours after the operation they all displayed maximum expansion of the melanophores.

Five pale animals, Nos. 11, 5, 50, 51, 54, were subjected to the operation of exposure of the brain without removal of the pituitary. On being placed in moist shady surroundings like the foregoing animals they became dark within 3 hours of the operation.

Five pale animals, Nos. 52, 92, 20, 22, 1, were treated like the preceding set, the optic nerves being in addition severed at the chiasma. Placed in moist, cool surroundings they displayed full expansion of the dermal and epidermal melanophores 3 hours after operation. All the foregoing animals were placed in glass containers with water in a cool room (10° C.) in shady surroundings. They were kept under observation for 3 weeks, the web being examined microscopically from time to time and the water changed regularly. Throughout this period the survivors remained uniformly of the coal black hue characteristic of normal frogs subjected to similar environment, the melanophores fully expanded and the xantholeucophores contracted. Three animals were killed for permanent preparations of the skin (Nos. 36, 5, 92) a fortnight after operation. Nos. 52, 20, 1 and 51 died a week after operation, remaining dark until death, when the characteristic *post-mortem* pallor supervened.

Five animals, Nos. 72, 75, 74, 79, 78, were deprived of the entire pituitary gland 4 days after the preceding operations. The frogs selected for the purpose were dark before treatment, the melanophores of the web being reticulate. The morning after operation all this set of animals were pale, the melanophores contracted to fine points and the xantholeucophores so extremely expanded that the frogs displayed a lemon yellow aspect. No. 78 died in this condition 4 days after the operation, following which the animals had been placed like the foregoing sets in glass containers with water on a dark



A

B

[Photographed by Mr. J. Chisholm, artist to the Animal Breeding Research Department.]

**TEXT-FIG. 2.**—Two frogs, 19 days after operation, kept in wet shady conditions at 10° C.  
**A.** Partially hypophysectomised (removal of anterior lobe only).  
**B.** Complete hypophysectomy.

background at 10° C. The remainder were kept in these conditions along with the animals of the other sets for a fortnight under observation during which they never regained the condition of melanophore expansion normally appropriate to such surroundings. (Text-fig. 2.)

This experiment conclusively shows that the pallor of hypophysectomised frogs is not due to disturbance of the C.N.S. occasioned by the operation, and that it is independent of the pars anterior. The frogs employed were *R. temporaria* (including *R. fusca*). But other experiments were carried out on *R. esculenta* with similar results. There is no need to describe further observations under this heading except to state that the whole pituitary gland was removed from thirty individuals with essentially the same result, namely that hypophysectomised frogs are unable to display colour adaptation, and remain permanently pale in natural surroundings (cold, wet, shady), which invariably induce melanophore expansion in the intact animal and also in individuals from which the anterior lobe alone has been removed, or the optic nerves severed at their origin, or the brain exposed in a manner identical with the procedure adopted for complete hypophysectomy. This conclusion is fully consonant with the experiments of Smith, Allen and Atwell who found that tadpoles remained pale after early ablation of the hypophysial *anlage*. We, however, are able for the first time to bring the results of hypophysectomy into harmonious relation with the effects of pituitary administration. Other experiments on the colour responses of the hypophysectomised frogs in response to normal stimuli will be recorded after consideration of the action of drugs.

#### § 6. *Action of Nicotine and Apocodeine on the Melanophores of the Hypophysectomised Frog.*

In the last paper of this series we pointed out that while the action of drugs upon colour responses in the frog favours the hypothesis of melanophore innervation, direct experiment by local nerve section and stimulation does not afford confirmatory evidence for this view. It was shown that whereas adrenaline, tyramine and ergotoxine induce expanded melanophores to contract, administration of nicotine and apocodeine to pale frogs in quantity adequate to ensure general motor paralysis results in a partial expansion described as "stellate" in contrast with the more pronounced "reticulate" condition which follows treatment with post-pituitary extract. It was suggested that, if the action of drugs can be accepted as indicating a nervous control of the melanophores, the negative results of local stimulation of nerve trunks might be due to the possibility that the melanophores are in normal life more or less continuously subject to what may be described as tonic

impulses promoting the contracted phase. In that case the darkening produced by moisture, cold and shade would not be due to inhibition of impulses tending to bring about contraction, but to some other co-ordinating mechanism, a suggestion further sustained by the time relations of colour response in the frog and the fact that darkening induced by natural factors is accompanied by the reticulate condition characteristic of pituitary administration.

Now the hypophysectomised frog provides us with fresh material for testing this possibility more intimately. For placed in those surroundings—cold, wet, shade—which are the optimum conditions for melanophore expansion, it remains pale. If, therefore, nicotine and apocodeine induce darkening in the hypophysectomised frog exposed to those natural factors tending to promote melanophore expansion in the frog which is still endowed with its pituitary gland, one would conclude either that conditions which normally induce darkening do not inhibit nervous impulses tending to contract the melanophores, or that nicotine, etc., act on the latter by some means other than peripheral nervous paralysis, *i.e.*, that nervous control of the melanophores, if it exists, is not significant to the rhythm of normal pigmentary response in the common frog.

For this reason eight frogs (A-H), hypophysectomised a week earlier, were injected intraperitoneally with nicotine and apocodeine as indicated in the accompanying Table. The animals were treated in the same conditions as they had been kept since operation, namely, in glass containers with water at a temperature of 8–10° C., in a shady situation. The condition of the melanophores was examined from time to time in the web. Two other hypophysectomised frogs were injected with 0.1 c.c. liquid sterile extract of ox pituitary for contrasting the “reticulate” condition in which the pigment granules form an apparently continuous network with the “stellate” or branching

Table II.

Treatment.	Condition of melanophores.			
	12 NOON.	1 P.M.	2 P.M.	5 P.M.
A 3 mgrm. nicotine	Contracted	Stellate	Stellate	Stellate
B 2   "   "	"	Stellate	Slightly stellate	Slightly stellate
C 1   "   "	"	Slightly stellate	Slightly stellate	Slightly stellate
*D 1   "   "	"	Contracted	Contracted	Contracted
*E 0.5   "   "	"	Contracted	Contracted	Contracted
F 15   "   apocodeine	"	Stellate	Slightly stellate	Slightly stellate
G 12.5   "   "	"	Contracted	Stellate	Stellate
H 10   "   "	"	Slightly stellate	Slightly stellate	Slightly stellate

\* No motor paralysis. (Injection at 12.0 noon.)

appearance of the pigment cells after motor paralysis with nicotine and apocodeine. At this low temperature it should be noticed that both effects were accomplished very much more slowly than in warmer conditions. The doses of nicotine and apocodeine administered were based on our previous observations in that connection.

While our memoir on the effect of drugs upon colour response in the intact frog was being prepared for the press, Kahn published an account of experiments recording the expansion of melanophores through the action of pilocarpine, based on the administration of doses ranging from 30-2 mgrm., and leading him to the conclusion that "diese Zellen unter der Herrschaft einer doppelten gegensätzlichen Innervation stehen." Kahn admits that it is not possible to produce melanophore expansion with other drugs of the same series (physostigmine, muscarine, for example), and his observations on pilocarpine are entirely in contradiction to others which we have recorded elsewhere. It is to be noted that some of his experiments were carried out on animals to which curare had been previously administered under conditions which were by no means above criticism. The author mentions that the curarised animals were wrapped in *moist* filter paper. We may add that we have not been able to devise a stimulus resulting in what is ordinarily described under the term excitement which will induce pallor in a dark frog exposed to optimum conditions for melanophore expansion, despite his and older observations to the contrary. Moreover, Kahn does not amplify his thesis by any observations indicating an opposing action by drugs of the atropine series, nor does he fully explore the possibility that the alleged effect of pilocarpine—which he describes after a considerably longer latent period than the adrenaline response in the opposite series—might be related to the mechanism of pituitary secretion. It cannot be too strongly emphasised that experiments designed to display the efficacy of experimental factors to induce expansion of melanophores should be carried out under optimum conditions for pallor; and, *mutatis mutandis*, the demonstration of melanophore contraction after experimental treatment must be made in optimum conditions for darkening of the skin. Researches which are not safeguarded by a clear appreciation of the normal melanophore reactions are of doubtful value. One very relevant factor in melanophore expansion in the frog is moisture; and the copious secretion of slime by the frog's skin after administration of pilocarpine should of itself compel us to scrutinise with care any tendency to darkening of the skin following such treatment. Kahn specifically states that he kept his frogs moist in moderate daylight.

However, as our previous experiments were all performed with one sample

of pilocarpine hydrochloride (Burroughs Wellcome), it was necessary to reinvestigate Kahn's claim with a second sample of the drug (Parke, Davis, and Co.). Five normal pale frogs received respectively 35, 20, 20, 10, and 3 mgrm. by intraperitoneal injection. The animals were placed for three days in a brightly illumined situation and white background in separate dry glass containers at a temperature of 10° C. Microscopic examination of the web showed that the melanophores were fully contracted at the beginning of the experiment in all cases. After half an hour they were uniformly pale and the melanophores were fully contracted. They were kept under observation for 9 hours, during which they remained uniformly pale, and microscopic examination of the melanophores in the web after 30 minutes, 1½ hour, 3 hours, 5 hours, and 8 hours displayed no expansion during the period of observation. We are thus unable to confirm Kahn's claim that pilocarpine induces melanophore expansion. The reader may judge whether the inference which he draws would legitimately follow, even if the observation were established.

§ 7. *The Action of Post-Pituitary Extract on the Skin of the Hypophysectomised Frog.*

An important link in the evidence pointing to a definite rôle for the posterior lobe of the pituitary gland in the normal colour responses of the common frog is provided by the fact that post-pituitary administration induces melanophore expansion in the hypophysectomised frog. As high temperature tends to promote pallor and low temperature darkening of the skin, it seemed advisable to test the minimal dose requisite to produce darkening at different temperatures. On the hypothesis that melanophore expansion in response to normal stimuli is the result of increased posterior lobe activity, it would be expected that at low temperatures an appreciable difference between the minimal dose for normal and pituitaryless animals would be found. The results obtained suggest that this is indeed the case.

Sixteen normal and sixteen hypophysectomised animals were placed in separate dry glass containers on a white background for 36 hours before the experiment. The pituitaryless frogs had been operated upon 5-7 days before injection. Six animals of each series were kept at 22-23° C. and ten of each series at 12-13° C. The doses are given in the Table annexed in equivalents of the liquid sterile posterior lobe extract, "Infundin" (Burroughs Wellcome), used in our previous experiments. It will be noted that there is an appreciably greater sensitivity of normal as compared with hypophysectomised frogs at the lower temperature (Table).



Table III.—Action of Post-Pituitary Extract on the Hypophysectomised Frog.

Normal series.			Hypophysectomised series.		
Dose in c.c.	Weight in grm.	Condition of melanophores 2 hours later.	Dose in c.c.	Weight in grm.	Condition of melanophores 2 hours later.
(1) 22° C.—					
0·00001	17	Contracted	0·00001	18	Contracted
0·00005	17	"	—	—	—
0·0001	22	"	0·0001	18	Contracted
0·0005	22	Reticulate	0·0005	18	Reticulate
0·001	24	"	0·001	20	Stellate
0·01	25	"	0·01	21	Reticulate
0·1	—	—	0·1	24	"
(2) 12° C.—					
0·000001	18	Contracted	0·000001	17	Contracted
0·000005	22	"	0·000005	17	"
0·00001	23	"	0·00001	21	"
0·00005	23	Stellate	0·00005	18	"
0·0001	24	"	0·0001	22	Stellate
0·0005	26	Reticulate	0·0005	23	Contracted
0·001	26	"	0·001	24	Reticulate
0·005	33	"	0·005	24	"
0·01	32	"	0·01	24	"
0·1	30	"	0·1	26	"

After the experiment water was added to all the containers, which were placed again in a cool room at 10° C. On examination of the web, three days later, it was found that the melanophores were expanded in all the normal frogs, while they were completely contracted in all the hypophysectomised animals with the exception of one of the two which received the highest dose of pituitary extract (0·1 c.c., 20 per cent.), and displayed a very slightly stellate appearance, which subsequently gave place to the characteristic condition of complete contraction of the melanophores.

Thus we may not only state that hypophysectomised frogs remain pale in surroundings which induce darkening in normal individuals, but that when caused to assume the latter condition by administration of post-pituitary extract they regain their characteristic pallor under exposure to influences which are inevitably followed by darkening in normal pale frogs.

### § 8. *Colour Changes in the Breeding Season.*

It has long been known that an intense darkening of the skin is characteristic of the frog in the breeding season. We have not had opportunity of observing actually the responses of frogs in this condition, but it is well to point out that it does not seem necessary to postulate any special explanation

for this phenomenon, since the breeding season, when pairing takes place round the shady margin of muddy ponds at a comparatively low temperature, is pre-eminently the time when large numbers of frogs can be seen in nature subject to the optimum conditions for melanophore expansion, namely, cold, wet, and relatively shady surroundings. Dr. Crew has called our attention to the fact that the bluish tinge noticed on the under surface of the male, especially at this period, is also very well seen in frogs into which pituitary extract has been injected. At the same time, it may be noticed that if further research reveals any direct relationship between posterior lobe activity, on the one hand, and general metabolic or special reproductive activity on the other, the evidence that colour response is controlled by post-pituitary secretion would suggest a possible mechanism for controlling other bodily processes eminently susceptible to seasonal influences.

#### *§ 9. Summary and Conclusion.*

Before attempting to outline an hypothesis of the mechanism of pigmentary response in the frog, the salient results of this and previous researches may be briefly summarised as follows:—

(1) The synchronous colour changes displayed by the common frog, resulting from the reciprocal "expansion" and "contraction" of the melanophores (dermal and epidermal), on the one hand, and xantholeucophores on the other, are determined by a variety of natural factors of which temperature, humidity, and light are among the more significant; warmth, dryness, and bright illumination tend to promote the pallor associated with melanophore contraction, while cold, moisture, and shade tend to induce the darkening of the skin resulting from melanophore expansion. These responses are gradual, and require periods of time, ranging from half-an-hour to several days, to reach their completion according—within limits—to the intensity of the incident forces.

(2) Extracts of the posterior lobe (*pars intermedia*) of the pituitary gland of mammals, birds, reptiles, amphibians, and fishes have a specific local action on the melanophores of the frog, inducing maximal ("reticulate") expansion both in the intact animal, in the isolated limb or in isolated strip of skin; this response is an extremely delicate indicator for post-pituitary secretion, and sufficient of the melanophore stimulant can be extracted from the gland of a single frog to induce darkening in more than fifty other individuals. The melanophore stimulant differs from the "pressor," and agrees with the "uterine" component in certain biochemical properties.

(3) While removal of the anterior lobe of the pituitary gland in the frog does not interrupt the normal sequence of pigmentary response to natural

factors, after removal of the whole gland, frogs remain uniformly pale, with complete contraction of the melanophores and maximum expansion of the xantholeucophores even in natural conditions which invariably induce darkening of the skin in controls. After injection of post-pituitary extracts, the characteristic melanophore expansion is evoked in hypophysectomised as in normal pale frogs, but the hypophysectomised frog subjected to such treatment regains pallor even when exposed to surroundings which represent the optimum conditions for darkening in the normal animal.

(4) The intravenous administration of sympathomimetic reagents such as adrenalin and tyramine evokes pallor in dark normal frogs, while nicotine and apocodeine, in quantity adequate to induce motor paralysis, induces a partial darkening, associated with a "stellate" appearance of the melanophores, both in normal pale and hypophysectomised frogs. Pilocarpine and atropine were not found to affect the melanophores of frogs exposed to optimum conditions for pallor and darkening.

(5) Neither section nor stimulation of peripheral nerve trunks were found to be followed by local pigmentary changes in the frog.

The body of new data, which we have presented in the series of investigations of which this forms the final contribution, will, it is hoped, enable the reader to appreciate the significance of the conflicting testimony of a large number of previous workers in this field. And it is legitimate, therefore, to review the present position and attempt a reinterpretation of pigmentary response in the frog.

The earlier workers directed their attention almost exclusively to the possibility of nervous co-ordination of colour change. And in fishes it is possible that this may be a significant factor. When we come to consider the Amphibia, it does not seem experimentally certain that there is a direct innervation of the melanophores. The evidence from peripheral section and stimulation is negative; while the opposing action of the reagents mentioned in § 4 of the above summary does not necessarily point to a direct nervous control of the pigmentary effector system, since, as Langley has pointed out, these effects may be vasomotor in their seat of action. For reasons stated elsewhere we regard this as unlikely. However, the crucial point is whether—assuming the existence of a sympathetic supply to the chromatophores—it is actually significant to the colour changes which occur in the normal life of the frog. There are several considerations which point to the conclusion that a nervous control is not significant. One is the negative results of nerve section and stimulation, though there may be possibly other ways of explaining this discrepancy. Another is the very protracted latent period and gradual unfolding of the response which has been emphasised earlier.

But more convincing indications are provided by the action of nicotine and apocodeine in the hypophysectomised frog. If nicotine and apocodeine induce stellate expansion by peripheral paralysis, then the possibility of producing this condition in a pale animal signifies that impulses tending to promote contraction have been interrupted; but, after removal of the pituitary, it is possible to test the action of these reagents on the skin in optimum conditions for melanophore expansion, since the hypophysectomised animal remains pale in all normal surroundings. When this test is applied, it is found that the stellate reaction can be evoked even in the optimum conditions for darkening, thus suggesting that the impulses which tend to promote melanophore contraction are not inhibited under the influence of natural factors which induce melanophore expansion in nature (*cf.* § 6).

It would appear, therefore, that if a direct sympathetic innervation exists, it is not significant to the normal rhythm of colour change in the frog. And we may now turn to the alternative possibility that the synchronous pigmentary responses of Amphibia are controlled mainly by endocrine agencies. On the basis of existing knowledge we must then enquire into the rôle of the pituitary, adrenal and pineal glands. The latter possibly bears some relation to colour response in the larval Amphibian (McCord and Allen, Swingle, Huxley and Hogben) though the work of Laurens does not support the conclusion that it plays a very significant part; but, as indicated in a previous contribution, there is little encouragement for the view that the pineal gland has any relation to the colour response in the adult animal. The experimental evidence we have presented points to the probability that colour response in the frog is determined by a balance between adrenal (medullary) and post-pituitary secretion. The question then arises whether either or both secretions fluctuate significantly in amount in correlation with normal pigmentary changes in the intact animal. Critical data in this connection are not easy to obtain; but two considerations reinforce the likelihood of a significant variation in pituitary activity, namely, the minimal dose experiment recorded in § 7, and the action of pituitary extract on the isolated limb. When a pale limb is severed, the melanophores remain contracted, but not apparently through necrotic changes since application of post-pituitary extract evokes the usual expansion. But the facility with which adrenalin is destroyed in the tissues established by several lines of investigation (and confirmed by the negative results of its peritoneal injection on melanophores) would suggest that there is insufficient pituitary secretion in the severed limb of a pale animal to induce melanophore expansion even when the adrenalin content has fallen appreciably. We may, therefore, proffer provisionally the hypothesis that the colour changes of the intact

frog are due to fluctuating activity of the post-pituitary secreting mechanism in response to natural factors such as temperature, light, humidity, or to the rapidity with which the melanophore stimulant of the pituitary secretion is eliminated under the influence of similar agencies.

If this hypothesis is correct, the frog's appearance is an indicator of its own state of pituitary secretion. In any case, a definite advance has been made in our knowledge of the activity of the pituitary in the intact animal. While extensive researches have been conducted on the action of post-pituitary extracts on mammalian vaso-motor and plain muscle responses, it is to be regretted that they have not yet been clearly brought into line with experiments on the removal of the gland itself; and as yet, therefore, little is known of the function of the posterior lobe in the normal life of the organism. The effects of hypophysectomy here recorded conclusively demonstrate that this gland forms an essential link in the chain of factors controlling pigmentary changes; and the part which it plays, as inferred from the effects of extirpation, harmonise completely with a physiological response which can be evoked by injection of its extract. Moreover, the hypothesis of fluctuating post-pituitary secretion here advocated, suggests a method by which other effects of pituitary secretion in the normal animal may be studied; and the activity of the gland controlled by stimuli which do not involve mutilation of the animal.

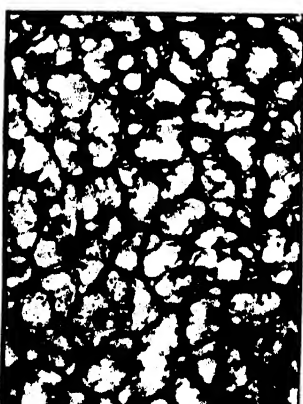
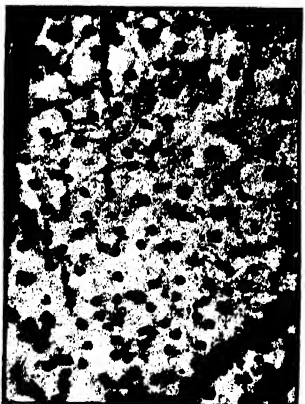
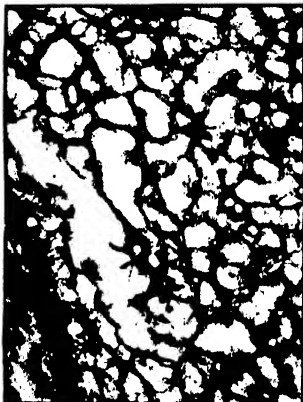
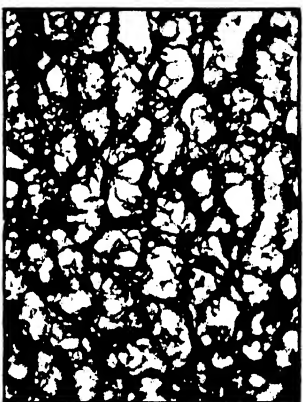
The authors are indebted to Prof. MacBride for kindly reading the MS., and to Prof. T. R. Elliott for invaluable criticism and advice in preparing the MS. for the press.

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DESCRIPTION OF PLATE.

Microphotographs of Melanophores in Web of the Foot. (By Mr. J. M. A. Chisholm, Artist to the Animal Breeding Research Department.)

1. Normal pale animal (dry, brightly illumined at 20° C.).
2. Hypophysectomised (both lobes), 19 days after operation (wet, shady, 10° C.).
3. Hypophysectomised (both lobes), after injection of posterior lobe (ox pituitary) extract, 6 hours later.
4. Normal dark animal (wet, shady, 10° C.).
5. Partially hypophysectomised (anterior lobe only), 19 days after operation (same conditions as 4).
6. Exposure of brain 19 days previously (same conditions as 4 and 5).
7. Hypophysectomised (both lobes) 5 days previously, 6 hours after 2 mgrm. nicotine (same conditions as 4-6).
8. Hypophysectomised (both lobes) 5 days previously, control (same conditions as 4-7).
9. Hypophysectomised (both lobes) 5 days previously, 6 hours after 10 mgrm. apocodeine (same conditions as 4-8).

*Studies in Amphibian Colour Change.*

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(Communicated by Prof. E. W. MacBride, F.R.S. Received February 14, 1923.)

[PLATES 2-4.]

I. GENERAL INTRODUCTION.

Colour changes in the Amphibia appear to have been first observed with anything approaching scientific exactness during the 18th century. Roesel von Rosenhof (1758), Pallas (1759), and Schneider (1799) are the most prominent among the first investigators. Following these early writers there have been many others, so that, at present, there are over 150 papers on Amphibian Colour Changes.\* It is impossible to summarise these papers here. This will be done under the different subjects investigated. The work done falls into three groups connected with the following problems:—

- (1) The character, inter-relationships and mode of reaction of the cutaneous effector organs† (chromatophores) responsible for the changes.
- (2) Influence of the various factors in the animal's environment, i.e., light, moisture, temperature and gases.
- (3) The nature of the control of the cutaneous pigmentary system.

\* For list of literature up to 1913, see Fuchs in Hans Winterstein's 'Handbuch der Vergleichenden Physiologie.'

† Hogben and Winton, 'Roy. Soc. Proc.,' B, 1922.



The present research is intended to give a statement of the existing stage of the investigations, and also to clear up certain of the controversial issues connected with the problems mentioned above.

Material has been obtained for these researches from *Rana temporaria*.

This research has been carried out in Prof. MacBride's laboratory at the Imperial College of Science. The author wishes to express his indebtedness to Prof. MacBride, and also to Dr. L. T. Hogben, for much valuable assistance and advice.

## II. METHOD OF "CONTRACTION" OF THE DERMAL MELANOPHORES OF THE FROG.

### 1. Introduction.

On the method of contraction of the melanophores in Amphibia there has been great diversity of opinion, and many theories have been put forward.

Some of the earlier writers—Brücke (6), v. Wittich (29) and Harless (14)—and later Bimmermann (5), Carnot (7), Fischel (12) and Hooker, maintained that the "contraction" is due to a withdrawal of the processes from the surrounding tissues. Hooker (16, 17, 18), in sections of lymph cultures, has observed processes partially contracted lying in spaces which he compares to lymph spaces.

Lister (21), Müller (22), and quite recently Dawson (9) have opposed this hypothesis for the following reasons:—1. There is no greater extent of *cytoplasm* in the cell-body during the "contracted" phase than during the "expanded" phase (21). 2. In *Necturus maculosus* there are occasionally stray granules in the neighbourhood of the "contracted" mass.

Biedermann takes an intermediate view—that the granules migrate inwards, and that this is followed by a contraction of the processes.

Kahn and Lieben in the frog (19), Spaeth in *Fundulus* (25), and Degner in *Praunus* (10) have shown, by photographs of consecutive "expansions," that the pigment granules are always present in the same tracks. Winkler, working on *Hyla* (28), and Ficalbi (11) oppose this, not finding the same minute repetition of processes that the others have.

Degner (10) in Crustacea and Ballowitz (2) and (3) in fish observe independent movements of the granules.

Ballowitz has further found nerve-endings on the processes of the melanophores. He also observes that the nucleus retains its position in all phases. This has been corroborated by Spaeth in *Fundulus* (25).

Schuberg, in 1903 (23), succeeded in staining the processes with Dahlia. He acknowledges that this is not always possible, but points out that in the

"contracted" phase he has obtained well-stained cell-bodies, and has been unable to find processes in these cases. This may be due to the fact that the processes are thinner than the cell-body, and therefore do not show the same intensity of staining.

## 2. *Methods.*

The following methods were employed:—

I. Observation of the web melanophores in the living frog.

II. Observation of the web melanophores of isolated skin in sterile lymph and Ringer preparations (Harrison's method).

III. Observation of the web melanophores in fixed preparations in expanded, stellate, or intensely "contracted" conditions. The skin was fixed in Bouin's solution, cleared in xylol, and mounted in balsam.

IV. Skin fixed in Bouin or Flemming, sectioned in celloidin or paraffin and stained in one of the following ways:—

- a. Eosin (in absolute alcohol and xylol).
- b. Hæmatoxylin (Delafields and Ehrlichs).
- c. Neutral red.
- d. Methylene blue and orcein.
- e. Picro-nigrosin.

## 3. *Observations.*

I.\* The migration of the pigment granules was very irregular. Some few were left behind. Others moved spasmodically as if hindered in their migration inwards. There was no sign of streaming similar to that in *Amæba*. Their movements suggested that they were being attracted towards some centre, but that fluid protoplasm, flowing outwards to take their place in the processes, slightly retarded and checked their inward migration.

II. (i) In the expanded condition of the melanophores, observations on the same cell at intervals of a day showed that frayed ends became blunt, and *vice versa*. In the first case they terminated bluntly further from the cell-body than the frayed ends had done originally, while in the second case the frayed ends were nearer the cell-body than the blunt ends had previously been (fig. 1, *a* and *b*).

(ii) At the blunt tips of the processes it was observed, in many cases, that the granules were massed together (figs. 1 and 2).

(iii) Two small protuberances appeared close together, as if growing out to meet each other (fig. 1, *c* and *d*). It is not certain that the processes were not there originally, and afterwards became filled up with melanin granules.

\* The Roman numerals refer to the methods of observation employed.

The original surfaces, however, from which these processes appear to have arisen showed no signs of "fraying" (fig. 1, c).

(iv) Many places were examined which at first seemed to show anastomosing of the processes, either of the same cell or of two neighbouring cells. Close observation ( $\times 1000$ ) always showed, however, that although the processes were often closely opposed, there was no anastomosing of the cell processes (figs. 1 and 2).

(v) Figs. 3-16 show the appearance presented by the melanophores in the stellate and "contracted" conditions. It was observed that even in the most extreme cases of "contraction" (figs. 12-16) there are always present a few isolated melanin granules in the neighbourhood of main "contracted" mass. Frequently they were in lines indicating the position of the processes as seen in the expanded condition (figs. 4-10, 12, 13, 17, and 20). In some few cases the processes themselves could be observed (figs. 8 and 9).

(vi) Some parts of the edges of the "contracted" mass were irregular and had not the smooth appearance characteristic of those parts which are presumably bounded by a cell wall.

III. In the fixed preparations the scattered granules were similarly observed in stellate and "contracted" melanophores (figs. 17-21). The drawings were made originally at a magnification of  $\times 1750$ .

IV. Sections of skin (celloidin method) showed exactly similar scattered granules as by Methods II and III. On the other hand, the processes were not stained to any degree of differentiation from the other tissues.

Sections of skin by the paraffin method were quite useless. The heating in the process rendered the material brittle and consequently the tissues were slightly displaced in the sections.

#### 4. Conclusions.

The presence of "frayed" ends, isolated granules, and irregular edges to the "contracted" mass (II (i), (v), and (vi)) militates against Hooker's theory and also that suggested by Biedermann. For, in prolonged and intense "contraction," the subsequent withdrawal of the processes postulated by Biedermann should be complete; so that the presence of even a single granule at any distance from the main "contracted" mass is inexplicable on his hypothesis.

Again, the observations of Lister and others, confirmed in the present research, regarding the irregular movements of the granules (I) can only be sufficiently explained by a theory which maintains the migration of the granules without any movement of the processes. The massing of the granules towards the tips, which often occurs in the most fully expanded

condition, would suggest that the movement of the granules is not merely passive, but active.

It is, therefore, certain that the various phases of the melanophore "contraction" and "expansions" are due to a migration of the granules and not to a movement of the cell processes. This being the case, it is proposed to substitute the words "concentrated" and "dispersed" for "contracted" and "expanded," respectively, in referring to these phases.

As to Hooker's actual observations, the fact that he used the paraffin method of embedding seriously detracts from the value of his observations. For, as mentioned above, slight displacement of the tissues always takes place, causing small fissures, which undoubtedly look like canaliculæ. Moreover, the processes observed in the present research (figs. 8 and 9) were in lymph preparations of isolated skin. This was due probably to the reduction of cellular pressures in the tissues causing a slight contraction of the surrounding cells away from the melanophores. Hooker's observations were made entirely from lymph preparations.

That the methods of staining, enumerated above, have failed to show any *differentiation* between the melanophore processes and the surrounding tissues also militates against the amoeboid theory. For had the processes been withdrawn from the "canaliculæ," the latter, being empty except for lymph, would appear *paler* than the surrounding tissues. It cannot be put forward that the tissues close in behind the retreating processes for Kahn and Lieben's experiments prove that if the cell does send out processes in the expanding phase then these processes do *not* push themselves indiscriminately in between the cells of the surrounding tissues.

It cannot be stated definitely from the observations above (II (iv)) that the melanophores do not form a syncytium. The evidence is purely negative; but until such anastomosing of processes has actually been seen and examined minutely the case for its existence must remain non-proven.

The observation recorded above (II (iii)) regarding the appearances of small protuberances towards the ends of the processes is open to the same criticism as Winkler's work. If that objection holds good in Winkler's case (as must almost necessarily be the case, in view of Kahn and Lieben's work) then it will hold good here also.

### III. NORMAL REACTIONS (LIGHT, TEMPERATURE, MOISTURE, BACKGROUND).

#### 1. *Introduction.*

Little controversy has centred round the colour responses of Amphibia to environmental conditions. This is due partly to the fact that the work done

has been carried out on different animals (adult and larvæ) and partly to the insufficient data given by many authors, thus making it impossible to compare results. In general it may be stated that for most amphibia it has been found that moisture, darkness, and low temperature, make for a dark condition of the animal; while dryness, light, and high temperature induce pallor. Hooker (17) on larvæ of *Rana pipiens* and Dawson on *Necturus maculosus* (9) have found that the reverse conditions exist.

In this research as many factors as could be expected to take part in colour change were introduced. So far only the dermal melanophores have been examined.

## 2. Methods.

*Rana temporaria* was used exclusively in these experiments. During the experiments the animals were kept in large accumulator jars which were sufficiently deep to prevent them from escaping. In the preliminary experiments\* they were kept in dipping jars. This may have caused a small amount of CO<sub>2</sub> to collect, but the results are too near the average for this to have produced any marked pathological symptoms. The following factors were taken into account:—Light and darkness, dryness and moisture, light and dark backgrounds, and temperature. Experiments were performed at three different temperatures, viz., 2° C., 17° C. and 27° C. These are average temperatures during a period of 6–12 hours, but they never fluctuated more than 2° C. in either direction. It may be noted here that no secondary reactions occurred within 12 hours.

It is impossible to tell accurately the condition of the melanophores in a given frog by ordinary observation. The number of melanophores per unit area varies considerably. So much so, in fact, that a frog with many melanophores per unit area in concentrated phase may appear darker than another with few melanophores per unit area in dispersed phase. It became necessary, therefore, for preparations to be made from each frog at the end of the experiments. It was possible to estimate fairly accurately the average condition of the melanophores in a particular frog. A series of photographs was made of melanophores in five different and equally spaced phases of concentration and dispersion. Assuming that dispersion occurs regularly (*i.e.*, its graph against time is a straight line) then numerical values can be given to these, having equal differences. This was done; the five values being –2 (extreme concentration) –1, 0 (stellate), +1 and +2 (extreme dispersion).† In order to eliminate any error due to personal observation, the preparations were examined several times. Where differences occurred in consecutive

\* These experiments are marked (p) in Tables I–III.

† These values are, of course, purely arbitrary.

observations on the same piece of skin, the case was carefully investigated. The errors, therefore, which occur, are due almost entirely to individual differences in the frog's responses.

### 3. Results.

Tables I, II and III give the results at 2° C., 17° C. and 27° C. respectively, together with the averages and probable errors of these averages. \*

Table I.

Conditions.	1.	2.	3.	4.	5.	6.	7.	Average.	Probable error.
(a) Lt + L + Lb + D	-1.5	-1.5	-1	-0.5	-1	—	—	-1.1	±0.10
(b) Lt + L + Lb + W	+1.5	+1.5	+1.5	+0.5	+1	—	—	+1.2	±0.13
(c) Lt + L + Db + D	-0.5	0	+1	+0.5	-1	+0.5	+0.5	+0.1	±0.17
(d) Lt + L + Db + W	+1	+1.5	+1.5	+1	0	+2	+2	+1.3	±0.18
(e) Lt + Dk + D	+2 (p)	-0.5 (p)	+1.5	+1	+0.5	+0.5	-0.5	+0.6	±0.23
(f) Lt + Dk + W	+1 (p)	-1 (p)	+2	+1	+0.5	0	+2	+0.8	±0.28

Table II.

Conditions.	1.	2.	3.	4.	5.	6.	7.	Average.	Probable error.
(a) Mt + L + Lb + D	-2 (p)	-2 (p)	-0.5	-0.5	-2	-1	—	-1.3	±0.21
(b) Mt + L + Lb + W	0 (p)	-2 (p)	-1	0	-1	-2	—	-1	±0.24
(c) Mt + L + Db + D	-2 (p)	-2 (p)	-0.5	0	-1.5	-1.5	—	-1.3	±0.22
(d) Mt + L + Db + W	+0.5 (p)	+1 (p)	+2	+1	0	+1	—	+0.9	±0.18
(e) Mt + Dk + D	-1 (p)	-2 (p)	-2	+0.5	-0.5	-2	—	-1.2	±0.28
(f) Mt + Dk + W	-1.5 (p)	-1.5 (p)	0	-1	0	0	—	-0.7	±0.21

Table III.

Conditions.	1.	2.	3.	4.	5.	6.	7.	Average.	Probable error.
(a) Ht + L + Lb + D	-1.5 (p)	-1.5 (p)	-2	-2	-1.5	-1	—	-1.6	±0.10
(b) Ht + L + Lb + W	-1.5 (p)	-1 (p)	0	+0.5	+0.5	-1.5	—	-0.5	±0.36
(c) Ht + L + Db + D	-1 (p)	+0.5 (p)	+0.5	+0.5	-2	-1	—	-0.4	±0.29
(d) Ht + L + Db + W	0 (p)	0 (p)	+1.5	+0.5	0	+1	—	+0.5	±0.17
(e) Ht + Dk + D	+1 (p)	-1.5 (p)	+0.5	0	-1	-1	-1	-0.5	±0.24
(f) Ht + Dk + W	+2 (p)	+2 (p)	+1.5	+1	+1.5	+1	—	+1.5	±0.12

Ht = high temperature (27° C.). Lt = low temperature (2° C.). Mt = medium temperature (17° C.). L = light. Dk = dark. Lb = light background. Db = dark background. D = dry. W = wet. (p) indicates experiments carried out in dipping jars.

It will be observed that on the whole, other things being equal, maximum dispersion is produced at low temperature, and maximum concentration at

\* Probable errors calculated from the formula,  $0.67 \sqrt{\frac{\sum d^2}{n(n-1)}}$ .

medium temperatures. Apparently higher temperatures do not have a further concentrating effect; if anything, they produce intermediate results, *i.e.*, a certain amount of dispersion as compared with the maximum concentration.

Moisture always produces greater dispersion (or less concentration) than do dry surroundings.

Light and dark backgrounds tend to produce concentration and dispersion respectively.

<sup>a</sup> The exact effects of light and darkness are not clear, as the background plays quite a considerable part in the reactions to light. It seems probable, however, from the figures that light produces concentration and darkness dispersion.

So far then, the results agree with those of other workers on Amphibia with the exception of the effects of high temperatures. There are, however, several results which require explanation.

With these opposing factors it is to be expected that intermediate colorations will occur under certain combinations of conditions. This is very marked in Table III, where the high temperatures have an intermediate effect. The conditions *b* and *c* both contain opposing influences, *viz.*, *Lb + W*, and *Db + D* respectively. It has been suggested by Cole (8) that high metabolic rate causes concentration and *vice-versa*. We should, therefore, expect the metabolism of the animal to make itself felt in the responses. This would particularly be the case where a delicate balance of the other factors existed. As already stated the possible errors consist almost entirely of individual differences in the frogs' responses. It is not surprising, therefore, that the probable error is much larger in these two cases (*b* and *c* Table III) than in cases *a* and *d*, where the factors—*Lb + D* and *Db + W* respectively—unite to cause concentration and dispersion respectively.

The diversity of coloration, under similar conditions, was particularly marked in those frogs which were being kept, as stock, in large white porcelain sinks. If all the pale frogs were removed one day, one was almost certain to find some more the next day. There may be a periodic change in the responses, but none was observed during the experiments. Further investigation is intended on this point.

The above explanation certainly does not hold for many of the results. It may be that all the factors relating to colour change have not been taken into account. On the other hand, the difficulty of observation, and the differences of frogs may sufficiently account for these high errors. Further refinement of observation and measurements are necessary to elucidate these problems.

As regards the time of reaction, a definite change in an animal towards its ultimate condition was usually observed within an hour. Even less time was

necessary under conditions *d*, Tables II and III, and in most of the low temperature experiments.

#### IV. REACTIONS TO GASES.

1. Cole (8) states that the effect of oxygen on *Rana clamitans* and *R. catesbiana* larvæ is to produce concentration of the melanophores. He concludes, further, that any effect which produces a higher metabolic rate in the animal will likewise tend to cause concentration. Uyeno (27) also comes to the conclusion that oxygen accelerates *post-mortem* concentration of the pigment, and that CO<sub>2</sub> acts in the reverse manner. Both these cases refer to the dermal melanophores.

2. In this research the following gases were employed:—oxygen, nitrogen, hydrogen, carbon dioxide. It was undertaken to determine whether any gases, by their own active influence or merely by the absence of oxygen, could affect the frog directly. It was thought, moreover, that it might throw some light on the normal responses. Thus the *whole animal* was always used. With the first three gases the frog was under their influence for 3 hours. CO<sub>2</sub> proved fatal within 15 minutes, even with quite a considerable amount of oxygen mixed with it.

3. (a) *Effects of Nitrogen\* and Hydrogen.*—Their effect was tried on both pale and dark frogs but in no cases did their conditions differ from the controls.

The frogs were darkened in water on a black background, and then transferred to dry, dark background conditions. Here the *rate* of paling was observed. For each of the pairs of frogs so used, careful observations of their reactions were made beforehand, so that the pairs were as nearly alike as possible.

(b) *Effect of Carbon Dioxide.*—This did not produce any effect, even when present in toxic quantities. It is little likely, therefore, to affect greatly the normal reactions.

(c) *Effect of Oxygen.*—In the case of oxygen, pale frogs were subjected to an atmosphere of oxygen on a white background, and dark frogs placed in oxygenated water on a black background. Observation on the former showed that no change of colour occurred. In the second instance, however, the frogs in oxygenated water became markedly paler after 1 hour. The treatment here was continued for 3 hours.

\* Since this paper was read, experiments have been carried out submitting frogs to absolutely pure nitrogen. This proves toxic in 1½–2 hours, but it shows that a certain amount of dispersion does occur. The mean for frogs in pure nitrogen is –0·6, while for the controls (conditions (a) Table II) the mean is –1·3. In the original experiments residual oxygen was probably present in the jar in which the frogs were confined.



## V. SUMMARY.

1. The presence of "frayed" ends to processes, isolated granules and irregular edges to the concentrated mass of granules precludes any theories postulating the amoeboid movement of the cell processes.

2. This is supported by (1) the irregular movements of the granules, (2) slight massing of granules towards the tips of processes in the dispersed phase, and (3) stained sections of skin.

3. No evidence was found for the supposition that the processes of the melanophores anastomosed and formed a syncytium.

4. The power of melanophores to extrude very small protuberances near the tips of the processes, lies open to too serious an objection to warrant its inclusion in a theory of melanophore response.

5. Adult *Rana temporaria* were found to respond, on the whole, similarly to other Amphibia, to the factors of normal environment.

Dryness and light background cause concentration.

Moisture and dark background cause dispersion.

Low temperature causes dispersion and medium temperature concentration. Higher temperatures appear to have an intermediate effect.

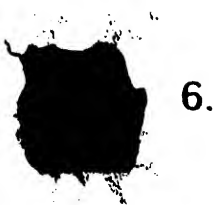
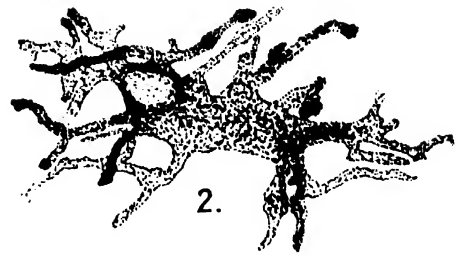
6. The effects of  $N_2$ ,  $H_2$ ,  $O_2$ ,  $CO_2$ , and  $Cl_2$  were investigated. Neither  $N_2$  or  $H_2$  produced any effect during a period of 3 hours.

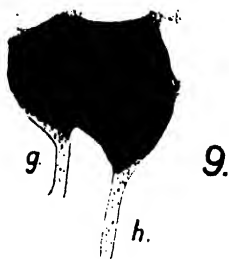
$CO_2$  did not affect the colour before proving toxic.

$O_2$  produced concentration in the melanophores.

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#### DESCRIPTION OF PLATES 2-4.

Figs. 1 and 2.—Expanded melanophores from isolated skin—

- (a) frayed end which became blunt after a few days.
- (b) blunt process became frayed.
- (c) two processes appeared here as shown in 1d.

Figs. 3-16.—Melanophores in stellate and concentrated conditions from isolated skin.

Figs. 8 and 9.—Melanophores in which the processes could be noticed at *e, f, g* and *h*.

Figs. 17-21.—Melanophores in stellate and concentrated conditions from balsam preparations.

Figs. 1-16 drawn from an original magnification of  $\times 1000$ . ('Voigtländer,' No. 8 obj. and d. comp. ocular.)

Figs. 17-21 drawn from an original magnification of  $\times 1750$ . ('Voigtländer' 1/12 oil imm. and d. comp. ocular.)

## *The Inhibitory Effect of Blood Serum on Hæmolysis.*

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### *Introduction.*

It has been recognised for many years that blood serum has an inhibitory effect on the hæmolysis produced by many substances, notably saponin and bile salts. Ransom (1), in 1901, observing that cholesterol inhibits the action of saponin, attributed the inhibitory effect of serum to the contained cholesterol. The quantities of cholesterol used in his experiments are far greater than those which occur in serum, and the experiments are inconclusive for that reason. Bayer (2), in 1907, investigated the inhibitory effect produced by serum on the action of the bile salts. He found that cholesterol has no inhibitory effect, that lecithin produces inhibition, but not in the quantities that occur in blood, and that the proteins of the serum are responsible for the inhibition. He calls attention to the results of von Eisler (3), who states that serum globulin inhibits the action of staphalolysin and of tetanolysin, and also those of von Liebermann, who finds that hæmolysis by soaps is prevented by serum albumin (4). Bayer's researches are, in the main, confirmed by Sellards (5). The investigations of Ludke (6) and of Scandaliato (7), who found that the inhibitory effect of serum is slightly increased after the injection of bile salts, may be mentioned. The conclusions of these authors are unreliable, since inadequate methods of measuring the amount of inhibition were used. References to various points in connection with the inhibition produced by serum *in vivo* and *in vitro* are to be found in the writer's earlier papers (8, 9, 10).

### *The Nature of the Inhibitory Substances.*

Before proceeding to the quantitative estimations, it is necessary to know which constituents of serum are responsible for the inhibition of saponin and bile salt hæmolysis respectively. Bayer's results might be taken as conclusive were it not for two considerations: (1) Bayer filtered most of the solutions of bile salts, and lecithin-bile-salt mixtures, whose hæmolytic power he wished to determine, through a Berkefeld filter, and thereafter tested their hæmolytic activity. He states that this procedure has no effect on the time taken for these solutions to produce hæmolysis. This is a

fallacy, for a solution of sodium taurocholate will not pass through a filter paper without losing some of its hæmolytic activity, while passage through a Berkefeld filter causes a very marked change indeed (10). It is therefore not permissible to regard the hæmolytic activity of a solution filtered in this way as identical with, or even corresponding to, the activity of an unfiltered solution; (2) Bayer used very rough quantitative methods—he refers to “slight hæmolysis,” “considerable hæmolysis,” etc., and, accordingly, would be able to detect only very marked degrees of inhibition. The same remark applies to the experiments of Sellards.

Accordingly, the experiments of Bayer have been repeated, using the more accurate methods outlined below. The results may be expressed as a series of conclusions:—

(1) Deproteinised serum exercises very much less inhibition on saponin and taurocholate hæmolysis than does serum before deproteinisation. Serum diluted 1 in 200 to 1 in 300 will give quite a measurable degree of inhibition; after deproteinisation, it will not inhibit in dilutions greater than 1 in 10. This result is attained whether the serum be deproteinised by the use of tungstic acid, by acetic acid and heat, or by simply heating or then filtering through a Berkefeld filter. Bayer's statement that the principal inhibitory substance in serum, when bile salts are used as hæmolytic agents, is protein in nature, is thus confirmed. Bayer makes no observations on the inhibitory effect of the proteins on saponin hæmolysis, nor did he observe that the protein-free filtrate has also inhibitory powers.

(2) As Bayer observes, euglobulin, pseudo-globulin, and albumin of serum, are all inhibitory.

(3) The digestion of the serum proteins by pepsin and trypsin, resulting as it does in the disappearance of the inhibitory property, must not be taken as direct proof that the proteins are the inhibitory substances. The inhibitory power disappears even while there is a considerable amount of protein still undigested. The true explanation is that the digestion results in the production of substances which accelerate saponin and bile salt hæmolysis, this acceleration balancing the inhibition caused by the remaining proteins.

(4) If the deproteinised serum be extracted with ether, the ethereal extract evaporated to dryness, and the residue be taken up in saline, the resulting solution will be found to have an inhibitory effect on saponin and taurocholate hæmolysis. The non-protein inhibitory substances are therefore ether soluble.

(5) If the ethereal extract be evaporated to dryness, and then thoroughly extracted with acetone, the acetone will be found to extract a substance



which inhibits the action of saponin on red cells, but which has no effect on that of bile salts. This substance is probably cholesterol. This observation confirms the statement of Ransom, with whom Bayer was unable to agree, that cholesterol inhibits saponin hæmolysis. On the other hand, Ransom considered that the inhibitory action of serum was due to the contained cholesterol, which is not the case, since the greater part of the inhibition is produced by the serum proteins.

(6) The substance not extracted from the ethereal extract of deproteinised serum by acetone has an inhibitory effect on the hæmolysis produced by sodium taurocholate. It has no effect on saponin hæmolysis. It is probably lecithin. Bayer recognised that lecithin suspensions inhibit the action of the bile salts, and his failure to find that the lecithin of serum acts similarly was due to the rough quantitative methods used. Suspensions of lecithin in saline are, of course, in a very different physical state from the lecithin in serum.

It thus appears that at least three of the constituents of serum are inhibitory substances. The proteins retard the hæmolysis produced by saponin, and also that produced by the bile salts; the cholesterol of the serum inhibits the action of saponin, while the lecithin inhibits the action of the bile salts. The degree of inhibition produced by the lipoids is very small compared with that produced by the proteins, since the latter are present in greater quantity.

It is desirable to estimate the inhibitory effect of serum quantitatively; this can be done by the application of methods devised for the quantitative study of hæmolysis in general.

In order to carry out this investigation, the methods given below have been used. The hæmolytic agents, with which the experiments are concerned, are saponin and sodium taurocholate; sodium glycocholate presents too many peculiarities to render it useful as a hæmolytic agent for quantitative work (10).

The saponin used in the experiments was Merck's pure saponin in most cases. Other specimens from other sources have also been used. The taurocholate used was also, in most cases, supplied by Merck, although pure samples from other makers, as well as samples prepared in the laboratory, were also employed.

#### *Method.*

It has been shown previously that the quantity of hæmolytic substance acting on a suspension of red cells, can be determined by the time taken for the production of complete hæmolysis of those cells at a given temperature (11). The technique used in this investigation is, in general, that described in the paper mentioned.

The standard blood cell suspension used is essentially a 5 per cent. suspension of human erythrocytes, four times washed, in 0·9 per cent. NaCl. Its preparation is described in the paper mentioned above. This suspension is always used within a few hours of preparation. The blood cells of the experimenter have always been used, and the suspension has not been found to vary appreciably, although prepared daily for three years. Further similar suspensions have been made from the blood cells of each of five other normal persons; these have proved almost identical, as regards the time taken for their hæmolysis by various dilutions of saponin, with suspensions made from the blood cells of the experimenter; it may therefore be taken that the suspension, if carefully prepared, is reliably constant, and representative of a suspension of red cells of normal persons. The estimations of the time taken for hæmolysis is carried out in a water-bath, made with glass sides, so that the condition of the contents of the tubes may be seen by transmitted light without removing the tubes from the bath. Behind the bath is a screen of white paper, ruled vertically and horizontally with fine grey lines at distances of 1 mm. apart, and lit by artificial light. Against such a background the end point, complete hæmolysis, can be most accurately gauged. The temperature of the bath is kept constant to within  $1/10^{\circ}$  C. The time taken for hæmolysis is registered by a stop-watch.

A series of stock dilutions of saponin are required. These dilutions are labelled 1 in 10,000, 1 in 20,000, 1 in 30,000, 1 in 40,000, 1 in 50,000, 1 in 60,000, and 1 in 70,000; intermediate dilutions can be made as required. The dilutions are made by dissolving a known weight of saponin in the necessary quantity of 0·9 per cent. NaCl. To facilitate calculation, each dilution is made 2·5 times the strength indicated; for instance, the dilution labelled 1 in 10,000 is really a 1 in 4,000 saponin solution; so that if 0·8 c.c. be taken, and have added to it 0·8 c.c. of saline or other fluid, and also 0·4 c.c. of blood suspension, thus making the final volume 2 c.c., the final dilution will be 1 in 10,000; in this way the dilution of hæmolytic agent actually in contact with the red cells is kept in round figures. The stock solutions of saponin keep unchanged as regards their hæmolytic power for about a week, if well stoppered and in a cool place.

The stock dilutions of sodium taurocholate are labelled 1 in 1000, 1 in 2000, 1 in 3000, 1 in 4000, 1 in 5000, and 1 in 6000; each being really 2·5 times the strength, as in the case of the saponin stock solutions. The preparation of a series of dilute solutions of this salt, which have a constant hæmolytic activity, constitutes the greatest difficulty in these experiments, and necessitates the greatest care. The following points are to be observed and are necessary for success:—(1) The specimen of taurocholate used must be pure and dried

carefully. The drying is carried out, *in vacuo*, over calcium chloride for several days. On no account should sulphuric acid be used as a dehydrating agent; the slightest contamination of the acid with the bile salt, such as is very liable to occur when the vacuum is being produced, results in the production of sulphurous acid which, condensing on the finely-divided salt, makes it variable and unreliable as a hæmolytic agent; (2) the taurocholate, after having been weighed in a stoppered weighing bottle, is dissolved in a suitable quantity of 0.9 per cent. NaCl to make a 2.5 per cent. solution. The water and the NaCl employed must be free from acid; the slightest trace of acidity renders the final solution unfit to use as a hæmolytic agent; (3) the 2.5 per cent. solution is diluted 1 in 10, thus making a dilution of 1 in 400, which, when 0.8 c.c. of it has added 0.8 c.c. of saline and 0.4 c.c. of standard blood suspension, will give a final concentration of hæmolytic agent, in contact with the cells, of 1 in 1000. From this 1 in 400 solution the other dilutions of the series are made, each being thus 2.5 times the concentration which they are used to produce; (4) the estimation of the hæmolytic activity of these solutions, and all experiments in which they are used, must be made immediately after. If the taurocholate be allowed to stand in these dilute solutions it undergoes a change of physical state, accompanied by a loss in hæmolytic power, and usually by the appearance of an opalescence in the solution (9). In a concentration such as 2.5 per cent. it will remain unchanged for some hours. It is therefore necessary to do all experiments immediately after the dilute solutions have been made; by observing this precaution it has been found possible to obtain consistent results, but the difficulties attendant on the use of sodium taurocholate as a hæmolytic agent for quantitative work cannot be overestimated.

All glass tubes, pipettes, flasks, etc., used in the experiments must be clean, especially when taurocholate is used as the hæmolytic agent. The cleansing is done with distilled water, followed by exposure to steam; all apparatus is then dried in a hot air oven. Pipettes and volumetric flasks are calibrated by weighing. Thermometers are calibrated by comparison with a standard instrument. The tubes in which the hæmolysis is carried out are of thin white glass, 7 by 1.5 cm. in size.

In order to find the quantity of hæmolytic substance inhibited by a quantity of serum, the following procedure is carried out. The times taken for the production of complete hæmolysis of a measured quantity of standard blood suspension (0.4 c.c. in these experiments), by various dilutions of hæmolytic agent, are determined, working at a constant temperature (25° C. as a rule). From these observed times, the curve relating the dilution of hæmolytic agent to the time for complete hæmolysis is constructed. This is

referred to as the time-dilution curve at  $25^{\circ}$ , or whatever the temperature may be. The curve at  $25^{\circ}$  C. is satisfactorily determined by the series of dilutions given above as "stock dilutions" in the cases of saponin and taurocholate respectively. If any one point falls off the curve, it is an indication that some error has been made, and the whole experiment is discarded. The imposition of this rule, though apparently leading to great loss of labour, by the necessity for care at every step, gives a confidence in results: as a matter of fact, such errors rarely occur after sufficient experience has been gained: the usual causes are either insufficiently cleaned tubes, or waiting too long after the making of the dilute solutions of taurocholate before carrying out the experiments.

To one of the less dilute solutions of hæmolytic agent—the best is 1 in 25,000 saponin or 1 in 2500 taurocholate—is added the serum whose inhibitory power is to be determined, after suitable dilution. It is satisfactory to dilute the serum 1 in 80 with saline. The contents of the tube prepared is, then, in the case of saponin as an instance—0.8 c.c. of the saponin dilution labelled 1 in 25,000, 0.8 c.c. of serum diluted 1 in 80, and 0.4 c.c. of suspension, added when the contents of the tube have attained the temperature of the water bath, the suspension itself being also at this temperature. In this way a saponin dilution of 1 in 25,000 acts on the red cells in the presence of 0.01 c.c. of serum, at the required temperature. The time  $T_1$ , taken for this dilution,  $d_1$ , to produce hæmolysis, will be longer than the time taken by it if it had been acting in the absence of the serum. Now by consulting the time-dilution curve previously obtained, the dilution  $d_2$ , which would take the time  $T_1$  in the absence of serum, may be found:  $d_2$  will obviously be a greater dilution than  $d_1$ . Converting  $d_1$  and  $d_2$  into concentrations in milligrammes,  $c_1$  and  $c_2$ , the number of milligrammes of hæmolytic substance inhibited by the serum will be  $(c_1 - c_2)$  milligrammes. This is, of course, based on the assumption that certain of the constituents of the serum form with the hæmolytic substance a non-hæmolytic compound, which plays no part in the subsequent reaction between the remaining hæmolytic substance and the red cells. That there is such a substance formed is an idea favoured by all the facts. Whether it is correct to assume that this substance, once formed, plays no part in subsequent reactions, will be considered later.

#### *The Inhibitory Power of Normal Human Serum.*

In order to obtain an idea of the degree of inhibition produced by the sera of normal persons, twenty such sera were examined. The following Table expresses the number of milligrammes of hæmolytic agent whose action was

neutralised by 0·1 c.c. of serum diluted 1 in 10. The hæmolytic agent was saponin in a dilution of 1 in 25,000, the experiment being carried out at 25° C., in the manner described above.

Serum.	Milligrammes of saponin neutralised.	Serum.	Milligrammes of saponin neutralised.
1	0·0423	11	0·0533
2	0·0566	12	0·0610
3	0·0467	13	0·0467
4	0·0455	14	0·0443
5	0·0465	15	0·0467
6	0·0625	16	0·0546
7	0·0616	17	0·0566
8	0·0650	18	0·0623
9	0·0616	19	0·0467
10	0·0556	20	0·0620

It will thus be seen that the inhibitory power of normal serum is remarkably constant, as would be expected from the fact that it is dependent on the protein constituents of the serum. The average inhibition is 0·0534 mgrm. of saponin by 0·1 c.c. of 1 in 10 serum.

Although the average inhibitory power has been tested in several abnormal conditions, no striking variation has been found.

*The Inhibitory Power of the Serum of Animals.*

This was investigated in order that a comparison might be made with human serum. The Table below expresses the results in the same way as the previous Table: the hæmolytic agent used was 1 in 25,000 saponin at 25° C.

Animal.	Milligrammes of saponin neutralised.	Animal.	Milligrammes of saponin neutralised.
Cat 1	0·027	Rabbit 1	0·035
2	0·022	2	0·027
3	0·030	Guinea-pig	0·037
4	0·043	Rat 1	0·031
5	0·032	2	0·023
6	0·067	3	0·030
7	0·030	Horse 1	0·043
8	0·042	2	0·052
9	0·037	3	0·050
10	0·039	Mouse	0·032

These figures, which are rather too few to justify the drawing of conclusions, indicate that while on the whole the inhibitory power of the sera of these animals is lower than that of the sera from man, they are nevertheless quite markedly inhibitory, 0·1 c.c. of the serum diluted 1 in 10 neutralising from 0·02 to 0·05 mgrm. of saponin.

*The Effect of Drying the Serum.*

In many hæmolytic investigations, it is very convenient to use sera dried by the method of Leers, or by other similar methods. Sera were, therefore, tested to see if the process altered in any way the inhibition produced. The drying was performed *in vacuo* in some cases, and in the incubator at 37° C. in others. The inhibitory power of the fresh and of the dry serum was then determined using a 1 in 25,000 saponin solution as hæmolytic agent at 25° C. The sera were all from either man or the cat.

Serum.	Milligrammes of saponin neutralised by 0·1 c.c. serum (1 in 10).	
	Fresh.	Dried.
1	0·0467	0·0346
2	0·0443	0·0326
3	0·0467	0·0346
4	0·0546	0·0385
5	0·0566	0·0400
6	0·0623	0·0443

It is very apparent that the drying causes a loss of inhibitory power. This occurs whether the drying takes place *in vacuo* (sera 4, 5, 6), or in the incubator. It is, therefore, not permissible to use dried sera for experiments of the inhibitory action of serum.

*Variation from Day to Day.*

In order to see if there were any diurnal variation in the inhibitory power, the sera of four normal persons were examined daily for two weeks. To reproduce the figures is unnecessary; it is sufficient to state that during this period no variation, except such as might be accounted for by experimental errors, occurred.

*Changes of the Inhibitory Power on Exposure to Air.*

It is important to use fresh, and if possible, sterile sera for all these experiments, for if a serum be exposed to the atmosphere for some time, changes, presumably due to bacterial action, occur in it, causing great variations in the amount of inhibition produced. These changes were observed from day to day in ten sera, kept at room temperature. The following figures represent the number of milligrammes of saponin whose action was inhibited by 0·1 c.c. of serum diluted 1 in 10, on successive days; the saponin employed for the estimations was 1 in 25,000, acting at 25° C.

Serum 1	0·0455	0·0467	0·0390	0·0455	0·0478
2	0·0625	0·0546	0·0592	0·0525	0·0525
3	0·0616	0·0592	0·0625	0·0643	
4	0·0423	0·0443	0·0390	0·0445	
5	0·0650	0·0616	0·0667	0·0643	
6	0·0455	0·0400	0·0400	0·0500	0·0520
7	0·0616	0·0546	0·0630	0·0525	
8	0·0566	0·0480	0·0346	0·0525	0·0525
9	0·0556	0·0500	0·0430	0·0370	0·0370
10	0·0467	0·0467	0·0443	0·0400	0·0430

These figures indicate that a specimen of serum does not retain for more than a short time its inhibitory power unchanged. That the alteration is due to bacterial changes is very probable, since there is always a change as soon as the serum becomes cloudy, and also since sera kept on ice or in sterile flasks, keep their inhibitory power unchanged for a long time. In making these measurements of the changes, it appeared generally that, coincident with the appearance of a cloud in the serum, there was a fall in the inhibition produced, but that after a day or two the inhibitory power seemed to increase, there being by this time a marked opacity in the serum. Such a sequence of events was not met with, however, in all cases.

#### *Inhibition to Saponin and Taurocholate.*

Since the amount of inhibition produced by serum largely depends on the contained proteins, whether the hæmolytic agent be bile salts or saponin, it would be expected that those sera which are most inhibitory to saponin hæmolysis would be also the most inhibitory to the hæmolysis produced by the bile salts. This is the case. Below, the inhibitory efforts of ten sera, all from cats, on the hæmolysis produced by saponin and on that produced by sodium taurocholate, are compared. The saponin used was in 1 in 25,000 dilution, the taurocholate being in 1 in 2500 dilution; the results are expressed in milligrammes of hæmolytic agent neutralised. The sera were all fresh.

Serum.	Milligrammes of saponin neutralised.	Milligrammes of tauro- cholate neutralised.
1	0·0140	0·25
2	0·0300	0·42
3	0·0400	0·48
4	0·0320	0·40
5	0·0670	0·65
6	0·0420	0·40
7	0·0230	0·45
8	0·0320	0·50
9	0·0370	0·48
10	0·0320	0·44

While it is generally true that a serum which powerfully inhibits the action of saponin has the same powerful inhibitory effect on bile salt hæmolysis, the parallelism is not quite complete; occasionally a serum will be found which is much less inhibitory than would be expected to one or the other of the hæmolytic agents. Except for the observation that the inhibitory power is not entirely determined by the proteins, but also to some extent by the lipoids of the serum, it is difficult to account for this fact.

*The Relation of the Quantity of Hæmolytic Substance Neutralised to the Quantity producing Hæmolysis.*

It has been seen that the effect of the addition of serum to certain hæmolytic agents is to lengthen the time taken by them to produce hæmolysis. In the case of sodium taurocholate or sodium glycocholate, the addition of serum causes a slight cloud to appear in the previously clear solutions; this cloud is the more marked the more concentrated the solution of hæmolytic agent used. It may well be that the cloud is occasioned by the formation of the non-hæmolytic substance; there is no opportunity of testing whether this be so or not, for it is impossible to filter off this fine precipitate, both because of its fineness, and also because it has been seen that the filtration of solutions of bile salts alters their hæmolytic power, the property by which their concentration may be most exactly determined. It is therefore necessary to determine the relation which exists between the time taken for a certain amount of hæmolytic agent to produce hæmolysis with and without the addition of serum, and to find therefrom by indirect methods the quantity acting on the cells and the quantity rendered inactive.

If the quantity of hæmolytic substance neutralised by a suitable quantity of serum be measured, it will be found to vary with the concentration of hæmolytic substance employed in the experiment. For instance, if 0.1 c.c. of serum diluted 1 in 10, be added to a 1 in 20,000 solution of saponin, the time taken for hæmolysis, measured at a certain temperature, and from the previously ascertained time-dilution curve at that temperature, the amount of hæmolytic substance acting on the cells, and also the amount whose action is inhibited, be calculated, it will be found that the quantity inhibited is greater than if the experiment had been performed with, not a 1 in 20,000 solution of saponin, but a solution more dilute, for example, 1 in 30,000. This fact may be readily demonstrated, and is very striking. It suggests that the serum does not inhibit the action of the hæmolytic substance by the formation of a molecular compound, but rather by the formation of a loose physical one, dependent on the concentration of the



hæmolytic substance; such an occurrence would not be unexpected, since both inhibitory agent and hæmolytic agent are in the colloidal state.

In order to discover the relation of the quantity of hæmolytic agent acting on the cells, and the quantity whose action is neutralised, the following experiments have been carried out. The time-dilution curve for the hæmolytic agent used is determined at a constant temperature. The amount of this substance, inhibited by a constant amount of serum, added to a number of different dilutions of hæmolytic substance, is determined also in the manner described above. The relation between the quantity of hæmolytic substance acting on the cells, as determined by the time taken to produce hæmolysis, and the quantity which is rendered non-hæmolytic by the serum, as determined by calculation, being thus obtained, the relation of the one quantity to the other may be arrived at. In order to make this clear, one experiment will be given in full.

Sodium taurocholate, acting on 0.4 c.c. of standard blood suspension, at 25° C.

Time-dilution curve determined by the following points:—

(1000, 0.8), (2000, 1.9), (2500, 2.6), (3000, 3.6), (3500, 4.5), (4000, 5.8),  
(4500, 7.4), (5000, 9.7), (6000, 24).

The curve is expressed in the usual way, the figures on the ordinate representing the time in minutes required for the production of complete hæmolysis, while those on the abscissa represent the number of cubic centimetres of fluid which contain 1 gram. of the hæmolytic substance, a figure represented by the symbol  $\delta$ . Time-dilution curve for sodium taurocholate acting in the presence of 0.1 c.c. of cat serum, diluted 1 in 10, on the same amount of cell suspension, at 25° C. :—

(1000, 1.2), (2000, 4.2), (2500, 9), (3000, 24).

Now, for the purpose of calculation, assume that the non-hæmolytic substance which results from the interaction of the serum and the taurocholate is insoluble, and therefore plays no part in the subsequent reaction between the hæmolytic substance which is not neutralised and the added red cells. The data may be drawn up in a tabular form:—

$\delta_1$ .	$c_1$ .	T.	$\delta_2$ .	$c_2$ .	$x$ .	$x$ (calc.).
1000	2.000	1.2	1400	1.430	0.570	0.574
2000	1.000	4.2	3400	0.588	0.412	0.411
2500	0.800	9.0	4900	0.408	0.392	0.388
3000	0.666	24.0	6000	0.333	0.333	0.332

In this Table  $\delta_1$  indicates the dilution of hæmolytic agent at the beginning of the experiment,  $\delta_2$  the dilution acting on the cells, as judged from the time required for the production of hæmolysis—in minutes T. The concentrations  $c_1$  and  $c_2$  correspond to the dilutions  $\delta_1$  and  $\delta_2$ . The quantity of hæmolytic substance inhibited by the serum is denoted by  $x$ , in milligrammes. The value of  $x$ , as calculated from the formula given below, is also given; it will be observed that the error resulting is very small.

The relation between the quantity of hæmolytic agent neutralised,  $x$ , and the quantity acting on the cells,  $c_2$ , is given by

$$x = Ac_2^{1/n},$$

the values of A and  $n$  being obtained by plotting  $\log x$  against  $\log c_2$ , the result of which is a very good straight line. In the above experiment the value of A used is 0.5, while that of  $n$  is 2.66.

This formula has been found to express the experimental results with accuracy. It is, nevertheless, to be borne in mind that the range of these experiments is necessarily a limited one; it is impossible to make accurate observations of the time taken for hæmolysis by very concentrated solutions of the saponins or bile salts, since the time is so short, while it is also impracticable to make observations with very dilute solutions, since dilutions of saponin greater than 1 in 70,000 take hours to produce hæmolysis. As soon as serum is added to the hæmolysing agent the limits become narrower still, owing to the great lengthening of the time required to produce hæmolysis. Within the limits of the experiments, however, the relation expressed above seems to hold.

Proceeding in this way, the constants in the equation have been determined in a considerable number of cases. Below are given some of the results, the first series when saponin is used as the hæmolytic agent, and the second series when the hæmolysis is produced by taurocholate of sodium. The values of A

**Saponin.**

Serum.	Animal.	$n$ .	A.	Errors.		
1	Man .....	2.00	0.280	-0.005,	-0.0065,	+0.0380
2	" .....	1.50	0.414	-0.005,	-0.0020,	+0.0220
3	" .....	1.38	0.445	-0.081,	+0.0100,	-0.0009
4	" .....	1.18	0.315	-0.000,	-0.0080,	+0.0120
5	" .....	1.38	0.455	-0.020,	-0.0040,	-0.0320
6	Cat .....	1.54	0.517	-0.012,	+0.0210,	-0.0010
7	" .....	1.125	0.899	-0.000,	-0.0200,	+0.0100
8	Rabbit .....	1.125	1.020	+0.007,	+0.0010,	-0.0000
Average .....		1.391	0.543			

## Sodium Taurocholate.

Serum.	Animal.	n.	A.	Errors.
1	Man .....	2·66	0·500	+0·0046, -0·0005, -0·0332
2	" .....	2·35	0·538	-0·0071, -0·0055, -0·0062
3	" .....	1·88	0·894	-0·0190, +0·0320, -0·0010
4	" .....	2·40	0·680	-0·0020, -0·0130, -0·0000
5	" .....	3·30	0·525	-0·0000, +0·0320, +0·0010
6	Cat .....	2·26	0·602	-0·0088, -0·0082, +0·0028
7	" .....	2·60	0·603	-0·0210, -0·0010, -0·0000
8	Rabbit .....	2·20	0·642	-0·0043, +0·0130, -0·0000
Average .....		2·44	0·648	

and  $n$  are followed by the errors which are occasioned by their use as a basis for calculation.

It may be concluded that the values of  $n$  are higher in the case of taurocholate than in the case of saponin, while there are no great differences in the values of  $A$ .

It will be noted that these measurements are based on the assumption that the non-hæmolytic substance formed by the combination of the serum and the hæmolytic agent plays no part in the reaction between the remaining hæmolytic substance and the red cells; such an assumption is not necessarily a correct one. If the non-hæmolytic substance is insoluble, being a compound such as is formed between saponin and cholesterol, then it might be considered as removed from the field of the reaction. If, on the other hand, as is more likely, it is an adsorption compound between the proteins of the serum and the hæmolytic agent, the assumption is incorrect. For compounds of this latter nature bear a relation to the concentration of the fluid surrounding them; since by adding red cells, and allowing them to be hæmolyzed by this fluid, thereby necessarily lowering the concentration of hæmolytic agent in it, the quantity of the hæmolytic substance adsorbed at the end of the experiment will be less than at the beginning, it is fallacious to consider the adsorption compound as completely removed from the reaction involving the hæmolysis of the cells; for it may act by giving up to the surrounding fluid some of the hæmolytic substance which was bound to it in an inactive state at the commencement of the hæmolysis of the added cells. The addition of red cells, for the purpose of measuring the quantity of hæmolytic substance not neutralised thus disturbs the conditions existing at the beginning of the experiment. Accordingly, the figures given above must not be taken as indicating the actual quantity of hæmolytic substance neutralised by the added serum, for such is not measured by experiments of the nature used.

Since the addition of cells may in this way affect the quantity of hæmolytic substance apparently neutralised by the addition of the serum, it would be expected that the addition of many red cells would have a greater influence than the addition of few cells. The effect of using various strengths of cell suspension on the constants of the above equation may therefore be studied.

*Effect of adding Various Quantities of Red Cells.*

Experiments were carried out in a manner similar to those described above to determine the quantities of hæmolytic substance apparently rendered inactive by a constant quantity of serum, and the relation of these quantities to the quantities acting on the cells. Such determinations were made, using the addition of (1) 0.4 c.c. of the standard blood suspension, (2) the same amount of a suspension 1.5 times as strong as the standard, and (3) the same amount of a suspension twice the strength of the standard. In this way the effect of the quantity of cells to be hæmolyzed on the constants of the equation was obtained. One experiment will be given in full.

Using sodium taurocholate as hæmolytic agent.

With standard suspension: time-dilution curve at 25° C., determined by the following points,

(1000, 0.4), (2000, 1.75), (3000, 2.9), (4000, 4), (4500, 5.5), (5000, 11),  
(5500, 18.5), (6000, 30).

With addition of 0.1 c.c. of cat serum, diluted 1 in 10, the curve becomes

(1500, 2.2), (2000, 3.7), (2500, 8.5), (3000, 35).

From these curves,

$$n = 2.5, \quad A = 0.56.$$

The errors occasioned by taking these values are,

$$+0.02, +0.001, -0.00, +0.013.$$

With a cell suspension 1.5 times as strong as the standard: time-dilution curve for the same hæmolytic substance at 25° C. passes through the points

(1000, 0.7), (2000, 1.9), (2500, 2.5), (3500, 6), (4000, 12), (4500, 24).

With the addition of the same amount of serum as above, this curve becomes

(1500, 2.2), (2000, 6), (2500, 18).

From these curves,

$$n = 2.5, \quad A = 0.49.$$

The errors are,

$$-0.001, -0.032, +0.032.$$

With a cell suspension twice as strong as the standard

Time-dilution curve passes through the following points, at 25° C., for the same hæmolytic substance,

(1000, 1), (1500, 1·7), (2000, 2·5), (2500, 4·5), (3000, 12·5), (3500, 40).

With the addition of 0·1 c.c. of the same serum diluted 1 in 10, the curve becomes

(1000, 1·6), (1500, 3·5), (2000, 18).

From these curves,

$$n = 2·5, \quad A = 0·47.$$

The errors attached are

$$-0·022, -0·015, +0·017.$$

From this experiment it may be concluded that the quantity of cells to be hæmolyzed makes no difference to the value of  $n$  and little difference to the value of  $A$ , within the limits of experiment. In carrying out these experiments it was hoped that by finding the values of  $A$  and  $n$  when different amounts of cell suspension were added, it would be possible, by extrapolation, to find the true values of these constants when the amount of cell suspension to be hæmolyzed was zero; the differences are, however, far too slight to allow of this being done. Such as the difference is, it indicates that when many cells are added and hæmolyzed—and, accordingly, when the hæmolytic agent surrounding them is most used up—the quantity of hæmolytic agent held in an inactive state by the serum is less than it appears to be when fewer cells are to be hæmolyzed, and accordingly less fall in the concentration of the hæmolytic agent surrounding the cells. This difference is what would be expected if the non-hæmolytic substance were an adsorption compound between the serum proteins and the hæmolytic agent; it would not occur if the non-hæmolytic compound were insoluble, and thus removed from the field of the reaction which results in the cell hæmolysis. Similar results were obtained from each of five other experiments of the same nature, different strengths of cell suspension being used. In all cases it was found that the effect of increasing the number of cells to be hæmolyzed was to lower the value of  $A$ , leaving  $n$  unchanged. This is shown on the following table.

With sodium taurocholate, at 25° C., as hæmolytic agent.

With suspensions less concentrated than the standard it becomes very difficult to judge the end point; this difficulty, together with the fact that hæmolysis is very rapid, makes observations with such a weak suspension unreliable. Suspensions more than twice the strength of the standard are also difficult to work with, as hæmolysis is so delayed. Accordingly, the above experiments cover the range over which accurate results are to be obtained.

Experiment.	Strength of suspension referred to standard.	Value of	
		A.	n.
1	1	0·54	2·3
	1·5	0·47	2·3
	2·0	0·45	2·3
2	1	0·63	2·2
	2·0	0·55	2·2
3	1	0·60	2·86
	1·5	0·56	2·86
	2·0	0·54	2·86
4	1	0·67	2·4
	2·0	0·61	2·4
5	1	0·63	2·1
	1·5	0·55	2·1

*Effect of various Dilutions of Serum.*

In all the above experiments the inhibition produced by a convenient amount of serum, 0·01 c.c., has been considered. Greater quantities render the hæmolysis slow; smaller quantities are insufficient to produce a well-marked inhibition. It would be expected that if a certain quantity of serum inhibits a certain amount of hæmolytic agent, twice the quantity of serum would inhibit twice the amount of the hæmolytic substance. Numerous experiments have been performed to ascertain if this is the case, using principally saponin as the hæmolytic agent, and investigating the quantity of hæmolytic substance inhibited by various amounts of serum, from 0·02 c.c. to 0·002 c.c. It has been found that twice the quantity of serum inhibits very nearly twice the amount of hæmolytic substance, but not quite; the quantity of hæmolytic agent neutralised per unit of serum is somewhat greater when only a small amount of serum is present. For instance, estimating the quantity of hæmolytic substance neutralised in a 1-in-20,000 dilution of saponin by various amounts of serum, the following figures were obtained:—

Quantity of serum (c.c.).	Milligramme of saponin neutralised, per 0·004 c.c. serum.
0·008	0·350
0·007	0·410
0·006	0·450
0·004	0·500

The consideration of this result will not be attempted at present, since further investigation is needed before the exact relation is obtained; it might be considered as a phenomenon similar to that met with when various quantities of cell suspension are added, as described above, and explainable on the grounds that the compound formed between the serum and the hæmolytic substance is influencing the reaction between the red cells and the hæmolytic agent which is not neutralised.

*The Inhibitory Effect of Hæmoglobin.*

Since serum albumin and serum globulin have the property of retarding the hæmolysis produced by saponin and by bile salts, such a property might be expected to be possessed by other proteins. Of these, the one of greatest importance in connection with hæmolysis is hæmoglobin.

Pure hæmoglobin was prepared by the method of Reid (12), and of Adair, Barcroft and Bock (13), from the blood of the guinea-pig and of the cat. In order that the samples of hæmoglobin might be free from lipoids, great care was taken to thoroughly extract with many volumes of ether. In the case of the guinea-pig blood, crystals were obtained, these were washed, and then recrystallised, so that all lipid material might be removed. The final samples of hæmoglobin therefore contained at most such small traces of lipid that any inhibition of hæmolysis could not be attributed to lecithin or cholesterol. In order to remove all remains of cell envelopes, the hæmoglobin was passed through a Berkefeld filter.

The final samples distinctly retard the hæmolysis by saponin and by taurocholate, as shown in the following experiment.

Saponin, acting on 0.4 c.c. of standard blood suspension at 25° C. Time-dilution curve,

(1000, 0.3), (2000, 0.6), (3000, 1.4), (4000, 2.4), (5000, 3.8), (6000, 8.5),  
(7000, 20).

With the addition of hæmoglobin, in such quantity that the amount of hæmoglobin in the tube exactly equalled the quantity which would be liberated from 0.4 c.c. of standard blood suspension if hæmolysed, the curve becomes,

(2000, 0.9), (3000, 2.4), (4000, 3.5), (5000, 6), 6000, 13).

The presence of hæmoglobin has an inhibitory effect, not very great, on the hæmolysis produced by saponin. In this way it acts like the proteins of serum; if the quantities of hæmolytic agent neutralised be expressed quantitatively, it will be found that the same relation exists between the amount inhibited and the amount acting on the cells, as does in the case of

inhibition by serum; the logarithm of the quantity inhibited plotted against the logarithm of the quantity acting gives a very good straight line.

Sodium taurocholate, acting on 0·4 c.c. of standard blood suspension, at 25° C. Time-dilution curve,

(1000, 0·75), (2000, 1·5), (3000, 2·5), (4000, 6), (5000, 18).

With the addition of hæmoglobin as in the case of saponin, the curve becomes

(1000, 1·2), (1500, 4·5), (2000, 15).

The inhibitory action of hæmoglobin on the action of sodium taurocholate is, therefore, very great. As in the case of saponin, the logarithm of the quantity neutralised plotted against the logarithm of the quantity acting on the cells gives a straight line. The hæmoglobin seems, therefore, to act in a manner similar to serum. It may be noted that the addition of hæmoglobin to taurocholate solution causes a cloud to appear, similar to the cloud produced when serum is added to the bile salts.

*The Influence of the Inhibitory Effect on Hæmoglobin on the Time-dilution Curve.*

The relation between the concentration of a hæmolytic agent and the time taken by it to produce complete hæmolysis at a given temperature is stated by Arrhenius to be  $cT = k$ , where  $c$  is the concentration of the hæmolytic substance,  $T$  the time, and  $k$  a constant (14). Substituting dilution for concentration, this relation will be expressed by a straight line through the origin. That such a relation does not hold for saponin, the bile salts, and other hæmolytic agents, has been pointed out in one of the author's earlier papers (11). Instead of a straight line, the result of plotting the dilution against the time required for hæmolysis is a curve concave to the time axis so that the time taken by dilute solutions to produce hæmolysis is much longer than would be expected from Arrhenius' formula. To give a formula for this curve is a difficult matter; the expression given in the previous paper fits the facts well, but is purely empirical. The appearance of the curve suggests that it might be logarithmic, but the formula of a logarithmic curve does not describe the results, although if the readings corresponding to very concentrated and very dilute solutions be ignored, it is a good approximation.

It is obvious that, since hæmoglobin has an inhibitory effect on the hæmolytic action of saponin and of the bile salts, and since hæmoglobin is liberated as the result of hæmolysis, the inhibitory effect of the hæmoglobin must be taken into account in any attempt to deduce the mode of



action of the hæmolytic agent on the cells from the time-dilution curve. Arrhenius' deduction that the action of the hæmolytic agent is a monomolecular one, since the time-dilution curve is a straight line, is incorrect, not only because the curve is not a straight line, but also because no account is taken of the inhibitory effect of the hæmoglobin which is liberated from the cells.

— Many observations point to the fact that saponin and the bile salts act on red cells by a chemical action on the envelope. The particular component of the cell attacked by the hæmolytic agent is a matter of debate; it may be that more than one component is acted on chemically. If, however, the hæmolysis of the cells takes place according to a monomolecular law, then, since the liberated hæmoglobin neutralises a quantity of hæmolytic substance proportional to the root of the quantity left unneutralised, the relation between the concentration of hæmolytic substance added to the cells and the time taken for hæmolysis at a constant temperature would be  $(c-x)T = k$ ,  $c$  being the concentration of hæmolytic agent added,  $T$  the time required for hæmolysis of the red cells,  $k$  a constant, and  $x$  the amount of hæmolytic agent neutralised by the liberated hæmoglobin. The general form of such a curve, it may be pointed out, is similar to the curve actually obtained by plotting time against dilution. The difficulties of finding the quantity of hæmolytic agent neutralised by the hæmoglobin are obviously great, since the amount of the latter substance is constantly varying, being small when a few cells only are hæmolysed, and great when complete hæmolysis is approached, and also since the quantity of hæmolytic agent whose action on the cells is inhibited is related to the quantity which is left unneutralised at any particular moment. Further investigations on the subject are being carried out.

#### *Summary.*

1. The hæmolytic action of saponin is inhibited by the proteins of serum, and, to a lesser extent, by the cholesterol. The action of the bile salts is inhibited by the proteins, and also by the lecithin of the serum.

2. A method for the estimation of the amount of inhibition produced is given.

3. Various observations of the inhibitory effect of serum are recorded; the inhibitory power is fairly constant in man and in animals, is altered by drying the serum, and is affected by bacterial action.

4. A quantitative study of the inhibition produced by serum is recorded, and the relation of the quantity of hæmolytic substance neutralised by the serum to that quantity which is unaffected, is given. All the facts point to

the inhibition being due to the formation of a loose adsorption compound between the proteins of the serum and the hæmolytic agent.

5. The inhibition produced by hæmoglobin on saponin and bile salt hæmolysis is considered, and the effect of such an inhibition on the time-dilution curve is pointed out.

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#### *The Reduction of Methylene Blue by Iron Compounds.*

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(From the Biochemical Department, Cambridge.)

Among the many papers dealing with the Schardinger (1902) reaction in milk, is one in which Römer and Sames (1910) have shown that if a small quantity of a freshly prepared 1 per cent. ferrous sulphate solution be added to boiled milk the mixture will then reduce the formaldehyde-methylene blue reagent of Schardinger. They also found that this reagent was not reduced by ferrous sulphate in aqueous solution, and if milk, to which had been added a small quantity of ferrous sulphate solution, was boiled for 30 minutes with an occasional shaking with air, the reaction was negative.

Morgan, Stewart and Hopkins (1922) have shown that hypoxanthine and xanthine can take the place of an aldehyde in the Schardinger reaction; the two bases being oxidised to uric acid. Since the enzymes which work with

the aldehydes and the purin bases are destroyed by boiling, it seems likely that if the power to reduce Schardinger's reagent can be restored by the addition of a little ferrous sulphate solution to the boiled milk, then an investigation of the rôle the iron is playing will possibly throw some light upon the mechanism of the reaction.

#### *Experiments with Boiled Milk.*

Römer and Sames in studying the effect of ferrous sulphate in boiled milk employed the formaldehyde-methylene blue mixture usually known as Schardinger's Reagent or "F.M.B." It is easy to show, however, that once the organic catalyst has been destroyed by boiling the milk, the aldehyde plays no part in the reaction.

#### *Experiment I.*

A mixed sample of boiled dairy milk was used, a quantity being placed in test-tubes open to the air. Bath at 60° C. R.T. = time for complete reduction.

Boiled milk.	Ferrous sulphate.	Methylene blue preparation.	R.T.
A. 5 c.c.	0.5 c.c. 1 per cent. solution	0.5 c.c. F.M.B.	25 seconds.
B. 5 c.c.	0.5 c.c. 1 per cent. solution	0.5 c.c. methylene blue	25 seconds.

The methylene blue solution was of the same strength as in the "F.M.B." solution.

Römer and Sames appear to make some point of the fact that when ferrous sulphate is added to milk and the milk then boiled, the power to reduce methylene blue is lost. There can be little doubt that this is merely because the ferrous salt is oxidised during the process of boiling and shaking with air. Ferric salts, as might be expected, do not reduce methylene blue.

#### *Experiment II.*

Conditions being the same as in Experiment I.

Boiled milk.	Iron solutions.	F.M.B.	R.T.
A. 5 c.c.	0.5 c.c. 1 per cent. ferrous sulphate solution	0.5 c.c.	25 secs.
B. 5 c.c.	0.5 c.c. 5 per cent. ferric alum solution .....	0.5 c.c.	Not reduced.
C. 5 c.c.	0.5 c.c. 1 per cent. ferric chloride solution	0.5 c.c.	Not reduced.

If copper sulphate solution be reduced by means of copper in the presence of acid, the solution reduces methylene blue vigorously. The addition of

copper sulphate does not restore the colour of the methylene blue, but a drop of ferric chloride solution will do so immediately. Similarly if chromium sulphate be reduced by zinc in the presence of acid the colourless filtrate (which has been warmed to remove hydrogen) will bring about reduction of methylene blue. The addition of chromium sulphate will not restore the colour.

The fundamental circumstance that ferrous ions do not reduce methylene blue is clearly due to the fact that the ferric ion oxidises the leuco compound, so that an equilibrium is established. The experiments which follow demonstrate that in what have become known as hydrolytic oxidation-reduction systems (of which the interaction between iron compounds and methylene blue presents an example) the formation of a compound from which the hydroxyl ion is only slightly or not dissociated is an essential factor.

### *Experiment III.*

Determinations were made of the reducing power of boiled milk in the presence of ferrous sulphate.

It was found difficult to obtain a good end point by titration with methylene blue. The following technique was therefore adopted. A measured quantity 5 c.c., of a solution of methylene blue (1 part in 1000) together with 1 c.c. of a freshly prepared M/3 (where M denotes mol. wt. per litre) ferrous ammonium sulphate solution, were measured into a fairly large test-tube. The cork closing the test-tube was provided with inlet and outlet tubes and was pierced with a third hole to admit the glass tip of a burette, which contained the boiled milk. Nitrogen freed from oxygen (by passing through alkaline pyrogallol) was bubbled through the solution until all the air had been displaced. The solution of methylene blue and ferrous ammonium sulphate was then heated just to boiling, and titrated with the milk until colourless. 3.0 c.c. of milk were required. Two other samples of mixed dairy milk gave the same figure. 100 c.c. of these samples of boiled milk, therefore (in the presence of excess of ferrous ammonium sulphate), will reduce 166.6 c.c. of 1 part in 1000 methylene blue solution.

### *The Constituents of Milk responsible for the Reduction.*

500 c.c. of milk were diluted with an equal quantity of water and dilute sulphuric acid cautiously added to precipitate the casein. The filtrate from the casein was boiled and again filtered. The filtrate was carefully neutralised with caustic soda solution and then evaporated to 100 c.c. and filtered. A portion of the filtrate showed a reduction of methylene blue on adding a little ferrous sulphate solution. The filtrate was then made slightly alkaline

with caustic soda solution and precipitated with a strong solution of calcium chloride. It was then filtered and the filtrate, after being carefully neutralised, was found to give no reduction of methylene blue on adding a little ferrous sulphate solution.

The precipitate of calcium phosphate and citrate was dissolved in a little very dilute hydrochloric acid, and then carefully adjusted to pH 6.9—7.0. This solution on adding a little ferrous sulphate solution was found rapidly to reduce methylene blue solution. It is, therefore, apparent that the reduction of methylene blue by boiled milk in the presence of ferrous sulphate is due to no enzyme but to the inorganic constituents present in the milk.

It is known (Bauer and Wieland, 1918) that ferrous sulphate in the presence of calcium carbonate or of sodium hydroxide will reduce indigo to indigo white. There was, therefore, every reason to expect that ferrous hydroxide would reduce methylene blue. This was found to be the case.

An attempt was then made to determine the presence of a stoichiometric relationship existing between ferrous hydroxide and methylene blue. The result showed that the reduction of methylene blue was strictly proportional to the quantity of ferrous hydroxide present in the solution. Whilst the methylene blue was reduced to its leuco compound, the ferrous hydroxide was oxidised to ferric hydroxide.

#### *Experiment IV.*

Standard solutions of M/1000 (where M denotes mol. wt. per litre) ferrous ammonium sulphate and M/1000 methylene blue were prepared. 20 c.c. of the freshly prepared ferrous ammonium sulphate solution were placed in a small flask (fitted as in Experiment III), and to this was added a measured quantity of the freshly prepared boiled out methylene blue solution. A current of nitrogen was passed through the flask until all the air was displaced. The contents were then gradually warmed until nearly boiling, and an excess of fairly strong (N/10) sodium hydroxide solution was added. This was done

Amount of FeAmSO <sub>4</sub> , M/1000.	Amount of M.B. M/1000.	Observation.
c.c.	c.c.	
20	8.0	Completely decolorised.
20	9.0	" "
20	9.5	" "
20	10.0	Just "decolorised."
20	10.2	Very slightly blue.
20	10.5	Slightly blue.
20	11.0	Blue.
20	12.0	Blue.

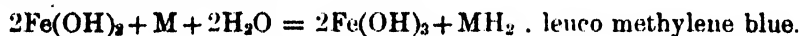
a number of times with varying quantities of the M/1000 methylene blue solution. Observations were made as to whether complete reduction took place or not.

*Experiment V.*

A few cubic centimetres of a saturated ferrous ammonium sulphate solution were placed in the flask, and a measured quantity of the M/1000 methylene blue solution was added. Nitrogen was passed through, as in the previous experiment, and the solution warmed until nearly boiling. No reduction occurred. The contents were then titrated with a freshly prepared N/10 sodium hydroxide solution, until the methylene blue was decolorised. The titration was repeated on varying quantities of methylene blue.

Amount of M.B. M/1000.	Amount of NaHO N/10 required.
c.c.	c.c.
10	0.45
20	0.80
25	1.00
30	1.20

These results show conclusively that a definite relationship exists between the amount of ferrous hydroxide and the methylene blue reduced. The relationship is expressed by the equation



On adding a drop of hydrochloric acid to the reduced methylene blue in the presence of the ferric hydroxide the blue colour is immediately restored, this is due to the oxidising action of the ferric ions.

*Experiment VI.*

The reduction by sodium bicarbonate and sodium carbonate solutions.

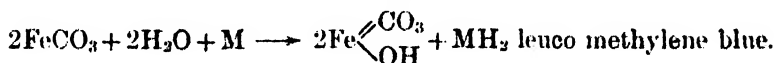
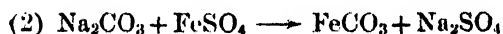
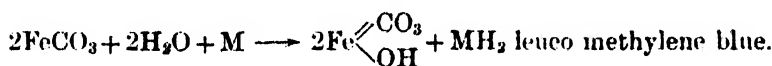
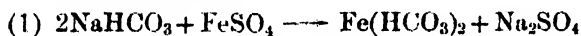
A. The sodium hydroxide of the previous two experiments was substituted by sodium bicarbonate solution, which also brings about reduction of methylene blue in the presence of ferrous sulphate. The following results were obtained.

Amount of M.B. M/250.	Amount of FeAmSO <sub>4</sub> M/3.	Calculated according to Scheme (1).	NaHCO <sub>3</sub> M/25 required to decolorise the M.B.
c.c.	c.c.	c.c.	c.c.
5	1	2.0	2.65 By the technique of Experiment V.
20	1	8.0	9.60 By the technique of Experiment V.
5	1	2.0	2.4 By the technique of Experiment IV.

B. With sodium carbonate instead of bicarbonate, the following results were obtained:—

Amount of M.B. M/250.	Amount of FeAmSO <sub>4</sub> M/3.	Calculated according to Scheme (2).	Na <sub>2</sub> CO <sub>3</sub> M/25 required to decolorise the M.B.
c.c. 5	c.c. 1	c.c. 1.0	c.c. 1.35
10	1	2.0	2.5
5	1	1.0	1.2
			By the technique of Experiment V.
			By the technique of Experiment V.
			By the technique of Experiment IV.

The end points in the titration were difficult to determine, and the titration figures were generally a little too high owing to the ionization of the basic ferric carbonate. It will be noticed that the sodium bicarbonate figures are almost exactly double the carbonate values. The results are in accordance with following schemes—



#### Experiment VII.

Reduction brought about by sodium phosphate, Na<sub>2</sub>HPO<sub>4</sub> . 2H<sub>2</sub>O.

Amount of M.B. M/250.	Amount of FeAmSO <sub>4</sub> M/3.	Sodium phosphate solution required.	Molecular strength of Na <sub>2</sub> HPO <sub>4</sub> .
c.c. 5	c.c. 1	c.c. 0.4	M/2
5	1	0.8	M/4
5	1	1.6	M/8
5	1	3.2	M/16
5	1	5.1	M/25
5	1	6.5	M/32
5	1	13.7	M/64
5	1	?	M/128
			End point approx.
			End point impossible to obtain.

The end points as dilution increased were more and more difficult to determine owing to the increasing ionisation of the ferric phosphate (and probable basic ferric phosphate) with dilution.

When acetic acid was added to the decolorised solution, the blue colour did not reappear. It returned, however, instantaneously on the addition of a little hydrochloric acid, showing conclusively that ferric ions are responsible for the oxidation of leuco methylene blue.

*Experiment VIII.*

Reduction brought about by sodium citrate.

Amount of M.B. M/250.	Amount of FeAmSO <sub>4</sub> M/3.	Sodium citrate M/6·25 required to decolorise the M.B.
c.c. 5	c.c. 1	c.c. 0·85 By the technique of Experiment V.
10	1	1·70 By the technique of Experiment V.
5	1	0·5 By the technique of Experiment IV.

The end points in the titration with sodium citrate were very difficult to determine owing to the production of a greenish colour. When the solution was completely decolorised it was then found that it would reduce more methylene blue, so that obviously an excess of sodium citrate was present. It is most probable that the last figure (0·5 c.c.) is the correct one.

Experiments qualitatively carried out showed that acetates and tartrates will effect a reduction of methylene blue in the presence of ferrous sulphate under anaerobic conditions. Toluidine blue and thionine blue act similarly to methylene blue.

It may be mentioned here that all these reactions proceed at room temperature under anaerobic conditions, but are more quickly effected at higher temperatures. The reduction, too, of methylene blue by ferrous hydroxide proceeds more quickly in alkaline solutions than in neutral.

The following experiments show the number of gramme-molecules of a ferrous compound which are required to reduce a definite quantity of methylene blue in the presence of excess of phosphates, etc.

Amount of M.B. M/250.	Salt in excess.	Amount of M/25 FeAmSO <sub>4</sub> required for decolorisation.
c.c. 5	Di-sodium phosphate ...	c.c. 1·15
5	Sodium bicarbonate .....	1·05
5	Sodium citrate .....	1·2
5	Sodium hydroxide .....	1·0



*Discussion.*

In the reactions of methylene blue with either sodium hydroxide, carbonate, bicarbonate, or phosphate in the presence of ferrous sulphate, it has been noticed that one molecule of methylene blue always reacts with two molecules of the ferrous compounds. Two hydroxyl ions are taken up by the two ferrous molecules, whilst the two corresponding hydrogen ions proceed to the methylene blue molecule.

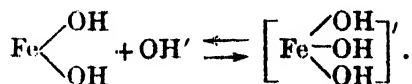
It is significant that in this reaction, involving both oxidation and reduction, water plays a most important part. In the accepted nomenclature methylene blue acts as a hydrogen acceptor and ferrous hydroxide acts as an oxygen, or rather, a hydroxyl acceptor. We have here a case almost strictly analogous with biological hydrolytic oxidations and reductions, with the important difference, however, that no thermolabile enzyme is present. Is it possible that this reaction can throw any light on the mechanism of the enzyme regulated hydrolytic oxidations and reductions?

Before answering this question it is essential to obtain a clear idea of the mechanism of the ferrous hydroxide-methylene blue system. We know that the ferrous ion appears to play no part in the reduction of methylene blue, hence we must deal with the molecule of ferrous hydroxide itself. Such a molecule we know becomes easily transformed to ferric hydroxide in the presence of an oxidising agent.

The clearest light we think is thrown upon the problem by the consideration of the ionic constants of ferrous and ferric hydroxides in aqueous solutions. The constant for ferrous hydroxide is  $1.64 \times 10^{-14}$  and that for ferric hydroxide is  $1.1 \times 10^{-36}$ , both at  $18^\circ \text{C}$ . ('Landolt-Börnstein Tabellen,' 1912). These figures indicate the high affinity that ferrous hydroxide must possess for a hydroxyl ion.

In picturing the mechanism of the reduction of methylene blue by ferrous hydroxide, three distinct schemes offer themselves, all of which are dependent on the extremely slight ionisation of ferric hydroxide.

In an aqueous solution containing ferrous hydroxide it is possible that not only will equilibria exist between hydrogen and hydroxyl ions, and ferrous and hydroxyl ions, but one also between ferrous hydroxide and hydroxyl ions with the production of a charged ferric hydroxide molecule, thus :

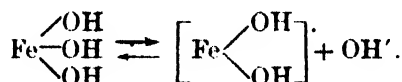


If such a molecule can transfer its electron to some acceptor it will produce neutral ferric hydroxide which (owing to its slight ionisation) can be

regarded as removed from the sphere of action. The reaction between ferrous hydroxide and hydroxyl ions, then, will proceed to completion in the presence of an acceptor capable of taking up hydrogen to form a slightly dissociated or undissociated compound. The amount of ferric hydroxide produced will be strictly proportional to the quantity of hydrogen acceptor reduced.

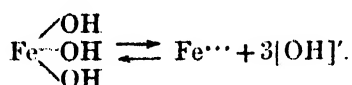
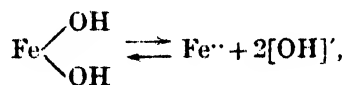
The system can be regarded in a slightly different manner.

Since ferric hydroxide dissociates to some extent, there will in all probability be the following equilibrium :



Hence, if the mechanism of the reduction of a hydrogen acceptor be regarded as the transfer of electrons from neutral ferrous hydroxide to the positively charged hydrogen compound of the acceptor, there will be produced not only the leuco compound of the acceptor, but the positively charged molecule of ferrous hydroxide. Since ferric hydroxide is only extremely slightly dissociated, the positively charged ferrous hydroxide will be removed almost entirely from the sphere of action.

Yet a third manner of regarding the system lies in an examination of the two equilibria :



If the mechanism of reduction lies in the transference of an electron from the ferrous ion to the positively charged hydrogen compound of the acceptor then the (neutral) leuco compound and a ferric ion will be produced. Since the dissociation of ferric hydroxide takes place only to a very slight extent, the ferric ion produced by electronic transfer will be removed almost entirely from the sphere of action and the reaction will proceed almost to completion.

It is difficult to decide which scheme is the correct one—it is possible that all play a part. Not, however, until further knowledge has been gained with regard to the precise details of the dissociation of ferric hydroxide can a correct decision be made.

Whatever scheme be preferred, the production of slightly dissociated

hydroxyl and hydrogen compounds is an essential factor. Were dissociation to occur to any considerable degree an equilibrium would be early established, and the mutual oxidation and reduction would not proceed far. (It is understood, of course, that the dissociation must be identical with that which led to its formation. Hypoxanthine, for instance, in the presence of an enzyme is oxidised to uric acid, which only dissociates hydrogen ions.)

It follows, therefore, that if we have two substances A and B, one of which is a hydrogen acceptor and the other is a hydroxyl acceptor, and whose compounds with the hydrogen ions and the hydroxyl ions respectively are only slightly dissociated, then A and B in the presence of water will be simultaneously reduced and oxidised. In other words, if A and B possess high affinities for the hydrogen ion and the hydroxyl ion respectively, such mutual oxidation and reduction will occur.

The mechanism of the oxidation of ferrous hydroxide in aqueous solution by means of oxygen is clear on this conception. The oxygen acts as a hydrogen acceptor, taking up the two positive hydrogen ions to form electrically neutral water, the corresponding two negative hydroxyl ions being immediately taken up by the ferrous hydroxide to produce the slightly dissociated ferric hydroxide and thus preserving electrical equilibrium.

The stability of the reduced and oxidised forms will depend on their power of ionisation into hydrogen and hydroxyl ions respectively. For instance, if into such a solution containing the reduced and oxidised forms there be placed another hydrogen acceptor capable of producing a reduced form, less dissociable than that of the former hydrogen acceptor, then the new hydrogen acceptor becomes reduced, and the reduced compound of the other oxidised. Oxygen is a good instance of this, for since it forms water which is electrically neutral it will then take up the hydrogen of any leuco compound so long as this is not entirely undissociated.

It is the high affinities, then, of methylene blue for hydrogen ions, and of ferrous hydroxide for hydroxyl ions, which afford, we think, the best interpretation of this hydrolytic-oxidation reduction system.

Turning to the consideration of enzyme regulated systems it is probable that a study of the affinities of the "oxygen acceptors" for the hydroxyl ions and their possible variations in the presence of enzymes, might help us to a clearer conception of the mechanism of these systems. Work on these lines is now in progress.

In the cases we have studied there seems to be no reason to assume the existence of the mechanism which Baudisch (1920, 1921) has suggested for the explanation of the reduction of nitrates to nitrites by ferrous hydroxide.

The reduction in that case appears not to proceed in neutral or faintly alkaline solutions unless oxygen is admitted to the system.

*Summary.*

1. The restoration of the power to reduce methylene blue to boiled milk by means of ferrous sulphate solution is shown to be due to the inorganic constituents of the milk.

2. It is shown that methylene blue is reduced by ferrous sulphate solution in the presence of sodium hydroxide, carbonate, bicarbonate or phosphate, and of the sodium salts of various organic acids, such as acetic, tartaric or citric. Ferrous sulphate solution alone will not effect any perceptible reduction.

3. The reduction has been followed quantitatively with the result that it is clear that two ferrous molecules always react with one of methylene blue.

4. The mechanism of the reduction has been studied with a view to throwing light on the biological hydrolytic oxidation-reduction system. It is suggested that the clearest light is afforded by the consideration of the relative affinities of the oxygen acceptor for the hydroxyl ion and of the hydrogen acceptor for the hydrogen ion.

Our thanks are due to Prof. F. G. Hopkins for his kind interest in this work.

One of us (E. J. M.) has worked with a grant allotted to Prof. Hopkins by the Medical Research Council; the other (J. H. Q.) is endowed by the Department of Scientific and Industrial Research.

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## *The Determiners of Cellulose Structure as Seen in the Cell-Wall of Cotton Hairs.*

By W. LAWRENCE BALLS, M.A., So.D. (Cantab.).

(Communicated by Dr. F. F. Blackman, F.R.S. Received March 28, 1923.)

(From the General Laboratory, Experimental Department, Fine Cotton Spinners and Doublers Association, Bollington.)

[PLATES 5 AND 6.]

This analysis of cell-wall structure as seen in cotton hairs began with the inference that diurnal growth rings existed therein. After this inference had been confirmed\* it was shown that spiral structures common to all the growth rings were present,† and that these were of at least two kinds, quick or "pit" spirals‡ and slow spirals corresponding to objects figured by De Mosenenthal.§ In our last communication we concluded that these two kinds of spirals might sometimes be independent of one another; but we now retract this conclusion in the light of results obtained by the improved micro-physical technique which we have substituted for the crude and difficult focussing method formerly employed. In the present communication we propose to study the origin of these spiral structures and to suggest some bearings of our results on problems relating to the molecular structure of cellulose. We abstain at present from extending our studies to comparisons with other plant cells.

Before proceeding to the analysis itself we may perhaps mention one side issue of this investigation which affects many calculations about cell-walls. The spiro-fibrillar structure formerly described evidently suggests that the wall is a sponge-like structure with (in the dry state) free air spaces therein. We have ample evidence that this is so, and that the specific gravity of cotton cellulose cell-walls, in their natural condition, is somewhere round 0.90 to 1.10, instead of having the value of 1.55 commonly accepted for the cotton cellulose itself; but the accurate determination of the mean cross-sectional area of the undamaged hair at any fixed temperature and humidity is a matter of

\* W. L. B., "The Existence of Daily Growth Rings in the Cell-wall of Cotton Hairs," 'Roy. Soc. Proc.,' B, vol. 90 (1919).

† W. L. B. and H. A. Hancock, "Further Observations on Cell-wall Structure as seen in Cotton Hairs," 'Roy. Soc. Proc.,' B, vol. 93 (1922).

‡ In a paper by C. R. Nodder, "A Study of Flax and Kindred Fibres," 'Journ. Textile Institute,' vol. 13, No. 9 (1922), the same spiro-fibrillar structure has been described for cotton, and related structure for flax and hemp. Our communication was received by the Royal Society before the publication of this paper.

§ De Mosenenthal, H. "Observations on Cotton and Nitrated Cotton," 'Journ. Soc. Chem. Ind.,' vol. 23, p. 292 (1904).

exceptional physical difficulty, and is being made the object of an elaborate separate investigation in our laboratory. Meanwhile, it is clear that no accepted values, nor calculations based thereon, are at all reliable.

### *Methods.*

*Biological.*—By the kindness of the Executive directors of our Association we were enabled in 1922 to grow successfully under glass a number of cotton plants (chiefly the Sakel variety of Egyptian cotton), and to mature a large number of normal ripe bolls from them, besides pickling bolls with acetic-acid absolute fixative at various stages of development. Some attempts to make definite "growth marks" on the hairs by modifying the environment of certain plants have been made, but without any particular success as yet. As a preliminary study we mapped the position and appearance of the nucleus at all stages.

*Physical.*—For the study of the material thus provided we have added to the techniques of swelling and pressure formerly described (*loc. cit.*) certain improvements to the microscope. The Spencer stand has an exceptionally good Spencer 4 mm. dry objective, supplemented by a Zeiss 1/8 inch water immersion and a Zeiss 2 mm. apochromatic oil immersion, with Zeiss 15 and 20 diameter eye-pieces and a Leitz oil immersion condenser, together with a revolving stage, in addition to the standard Spencer equipment; while a right-angled prism has been substituted for the plane mirror to avoid double reflections. This instrument is placed at the end of an optical bench which is illuminated by a Pointolite 100 c.p. lamp and carries a condensing telescope objective, a rack of Wratten and Wainwright's colour filters, iris diaphragm, Nicol prism and three revolvable quartz plates ( $\frac{1}{4}$ ,  $\frac{3}{8}$ ,  $\frac{1}{2}$ ), for producing elliptical polarisation and interference colouring, while a second Nicol is inserted in the microscope draw-tube just over the objective.

By the use of polarised light in this way, with Nicols fixed semi-permanently in the extinction position, by modifying the illumination by the quartz plates and colour screens, and by revolving the object, a wide range of effects is obtainable. A protractor fixed to the microscope body over which swings a pointer fixed to the eyepiece in which is a parallel-ruled graticule, enables angles of inclination of observed spirals to be determined.

*Micro-chemical.*—For an important link in the chain of methods we are indebted to a recent publication by Prof. Priestley,\* who had independently reached the same conclusion as ourselves, namely, that the primary wall was

\* Priestley, J. H., "Physiological Studies in Plant Anatomy.—IV. The Water Relations of the Plant Growing Point," 'The New Phytologist,' vol. 21, No. 4 (November 7, 1922).

not ordinary cellulose but rather "pre-cellulose." This wall in young cotton appears structureless, even in polarised light, but by boiling for half a minute in 10 per cent. KOH, then staining with Congo Red or Naphthamine Blue, and examining in polarised light, it is found to have a definite structure. We had previously experimented with various adsorption methods in order to bring out the presumed structure, but with very partial success until Priestley's discussion gave the clue to a method which is akin to the development of a photographic plate. It should be noted that even after such "development" with KOH we could at first detect no structure in the primary wall without the use of polarised light, nor could this be done conveniently without using the quartz plates, probably on account of the tenuity of the wall. Eventually a faint differential staining was made visible in ordinary light.

*Manipulation*—Immature cotton hairs are so packed on the seed that it is very difficult to extract any one hair separately, complete from base to tip, without breakage, especially before secondary thickening has begun. Once isolated, it is best to fasten each end to a separate cover-slip with a suitable cement, and after any required treatment to pull these two slips gently apart until the hair is stretched taut before applying a long cover-slip above both. Any bends in the hair much increase the difficulty of study. The cement used has commonly been celloidin, but for the KOH boil we have found rubber solution good enough.

*Cognate Researches.*—We owe much to the stimulus of our chemical and physical colleagues, Dr. Mary Cunningham and Mr. F. P. Slater, with whose researches our own are closely related. In this communication, however, we have purposely refrained (except in the one instance of the space-lattice) from carrying speculations any further than is warranted by observations suggested and conducted in our own laboratory. A joint communication may be feasible later. Mr. H. A. Hancock has conducted practically all the manipulation, preliminary observations and repetitions involved, and has contributed several useful suggestions.

#### *Results of Observations.*

In order to keep the sequence of these communications, we shall present the results according to the order in which they were obtained, rather than by following the ontogenetic sequence of cell development. This has the further advantage of concluding the present communication at a stage where the problem seems definitely to become one of space-lattice conformation.

*Relation of Convolution to Wall Structure.*—In the previous communication (*loc. cit.*) we stated a strong presumption that the pattern and direction of

the externally visible convolutions were determined mainly by the internal\* spiral structure of the wall, then visible with difficulty only. This conclusion we now confirm and amplify. If we take living uncollapsed hairs, which have formed an appreciable thickness of secondary wall, and map the reversals of spiral structure (e.g., in polarised light), then kill with alcohol and allow to dry with the hair freely suspended by one end, the convolution-reversal-map corresponds exactly to the spiral-reversal-map, excepting when the latter reversals may happen to be too close together to allow room for a convolution to take shape. Evidently the divergences between the two maps, on which we have previously commented in naturally ripened cotton, depend on accidental local deformations of, and restraints upon, the dying hairs. If the two maps are compared it is found that the relation illustrated by fig. 1 is almost invariable. Consideration of these diagrams, and of models, shows that the anisotropic structure of the cylinder is weak in the AB direction, more rigid in the

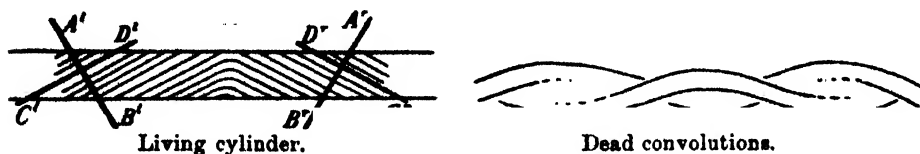


FIG. 1.

CD direction. Further, this weakness at right angles to the fibril axis is in some way linked to the desiccation of the cellulose; for a live hair which has been plasmolysed remains cylindrical even in alcohol until it is allowed to dry up. Evidently the loss of constructional water is irreversible, since a hair once fallen into convolutions cannot, by any method yet known, be again distended into a cylinder, even though the cylindrical form is not dependent on distension by osmotic pressure. The effect of strong NaOH (mercerising) may perhaps be mistaken for such a return to the living cylindrical form, but this is illusory. We consulted our colleagues as to the possible reagents which might inhibit this irreversible change, and a tentative success was obtained with  $\text{ZnCl}_2$  in 5 per cent. solution. Living hairs killed in this could be dried without collapsing, but the matter has not been further studied at present.

*Relation of Wall Structure to Polarised Light.*—Previous work on this

\* Some anomalous non-reversing spirals can be observed on the outside of the hair under low powers, which may belong to the cuticle. They are not dealt with further at present.



relationship, *e.g.*, by Harrison,\* has chiefly connected these phenomena with internal stresses, but examination of the living hair puts the question on an entirely different footing. Such hairs, provided that secondary thickening has begun (*i.e.*, on the 29th or 30th day in our greenhouse material), show the spiral structure everywhere fairly clearly, even in ordinary light; but when illuminated by plane polarised monochromatic light, with the plane of polarisation either parallel to the axis of the hair, or at right angles, every reversal of spiral is picked out by a dark band, *i.e.*, at the place where the fibrils run momentarily parallel to the axis (Plate 5, figs. 8 and 9). If the quartz plates are used to produce elliptical polarisation and consequent interference colours from white or other impure light, the right- and left-handed spiralled areas may be made to appear respectively scarlet and green, or blue and yellow, etc., and their mapping can be effected under a low power of the microscope without any fatigue or subjectivity; whereas such mapping has formerly required careful focussing under high powers, with a large risk of error in any obscure areas of the wall. Wherever a definite reversal of pit spiral has been shown in ordinary light the colour reversal has appeared in elliptically polarised light (Plate 5, figs. 6 and 7).

After examining uncollapsed hairs, dead or alive, the same method is easily used on convoluted hairs, provided only that these are kept quite straight. With the quartz plates suitably set (fig. 10), or, less easily, with the Nicols only (fig. 9), the minor effects due to the convolutions can readily be differentiated from the major structural effects on which the former are superposed. Moreover, it is evident that the variations of surface form and refraction in such a structure as we now know the hair to be are, in themselves, competent to produce the so-called stress effects, and the normal existence of such internal stresses becomes quite unlikely.

While endeavouring to reduce the visibility of these minor effects by swelling the hair with even 20 per cent. NaOH, it was noticed that the structural effects were not markedly affected, whereas swelling to an analogous extent with sub-critical strength  $H_2SO_4$  abolished all structural double refraction whatever. The chemical significance of this observation has yet to be studied, but again it indicates that the amphoteric character of cellulose is connected with a polarised structure in the unit aggregate akin to, though not necessarily identical with, that already suggested by the water-death-change.

The appearance of transverse sections of these hairs under these illumina-

\* Harrison, W., "Investigations on Textile Fibres," 'Roy. Soc. Proc.' A, vol. 94 (1918).

tions present certain features as yet unexplained. Whereas the extinction, or interference colour, should be uniform all over any one section if truly transverse, and should change only from section to section according to the direction of the spiral therein, yet the usual appearance approximates, very roughly, to bilateral symmetry. Sometimes a hard boundary runs radially between the two colours or degrees of extinction corresponding to a slow spiral slip plane (*vide infra*), and generally there would seem to be little doubt that the stresses produced by the razor edge have seriously altered the structure. For this reason we are unable to depend to any extent on even our own determinations of hair cross-sectional area as made by section cutting methods.

The brightness of the colouring with the quartz plates seems to depend mainly on the thickness of the cell-wall, but the nature of the change in the interference colours at the reversal point is a matter of importance for the general theory of cellulose structure. While the change over with the bend of the spiral is generally well defined, as already described, yet closer examination frequently shows islands or interlocking promontories of alternative colour. These seem to be due to interference and are therefore invisible in longitudinal sections, when only a single thickness of wall is examined.

To avoid the confusion introduced by the passage of light through both sides of the hair, we managed to obtain longitudinal sections of the mature hair at reversal points by methods formerly described.\* When only one thickness of wall is thus presented for study under plane polarised monochromatic light, we not only find the dark band effect at the reversal point, but, in addition, either side of the reversal can be extinguished completely by rotating the hair axis through a certain angle, while both halves are equally illuminated in the positions ( $0^\circ$  and  $90^\circ$ ) which also give the dark band (Plate 6, figs. 11, 12 and 13). The following data for one section are typical:—

Inclination of pit spirals to hair axis .....	24°
Inclination of hair axis to plane of polarisation in order to extinguish	
(a) Left-handed spiral .....	22° left.
(b) Right-handed spiral .....	22° right.

This is shown diagrammatically in fig. 2. Thus, if we regard the cell itself as the structural unit, there seems to be a reversal of optical properties,

\* With immature hairs (1-3 growth rings) excellent results were obtained by chloroform-paraffin embedding, celloidin being avoided altogether.

and not merely a reversal of fibril direction; and the structure on one side of a reversal point seems to be the mirror image of that on the other side.

This does not in itself prove the existence of stereo-isomeric forms of

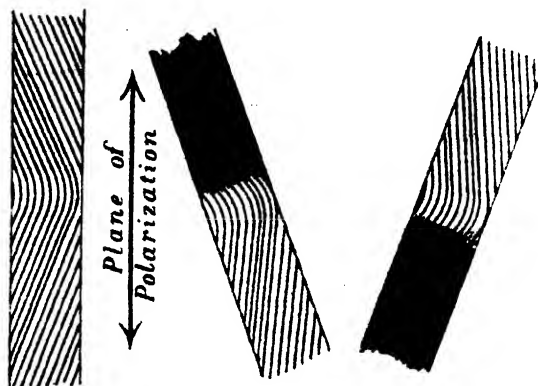


FIG. 2.

cellulose, though it suggests it. The effect might be produced by a change in the orientation of optically active units. It should however be noted that there are four positions of extinction for these secondary wall longitudinal sections (indicating a biaxial optical structure like that of rhombic, mono, or asymmetric crystals),\* and, when we further examine the inter-relations of the four extinction positions for right-handed and left-handed spirals separately, we find that these positions are constantly related, not to the direction of the cell-axis but to the direction of the fibril axis, regardless of whether the spiral is right-hand or left-hand. Thus the cell is not the constructional unit in the molecular sense, but the fibril is.

So far we have no evidence for differing molecular orientations within the fibrils of the right- or left-hand areas, nor for stereo-isomerism, and this in spite of the mirror-image structure of the visible wall, which results from the constancy of the pit-spiral angle.

*Slow (Sip) and Quick (Pit) Spirals.*—As stated in the introduction, we retract our previous conclusion that these two kinds of spiral do not invariably run in opposite directions. By using polarised light to locate the quick spirals, and examining special material in which slow spirals have been abundantly developed, we have now satisfied ourselves that our previous admission of exceptions to the rule was due to an excessive care

\* It may here be pointed out that clear microscopic definition of a cotton hair is not possible in ordinary light, unless an analyser be employed to cut out either the ordinary or the extraordinary rays.

in weighing the subjective error against, rather than for, an attractive hypothesis. The special material used is readily obtained from any much-used piece of cotton string, the older the better, and from some samples of druggist's "cotton wool," while severe twisting of a small bunch of hairs will develop the structure in any cotton. When immature hairs are thus twisted the operation should be done under water. Hairs from such material show innumerable slow spirals, especially when stained with iodine, much resembling the "slip planes" in timber described by Robinson,\* and similarly developed by stress, though probably by torsion in tension rather than by simple shear. Shear-developed ones are shown in fig. 18. Seeing that Robinson's slip-planes are figured as changing their position on arrival at a middle lamella, it seems probable that they are also pre-existing spiral structures† and may even be directly analogous to our slow spirals. Further, his photographs show them taking a spiral course,‡ but differing from the slip spirals of cotton in that both right- and left-hand spirals occur in the same cell; probably a fundamental difference in the molecular structure of wood and cotton celluloses is here made manifest. The analogy seems sufficiently good to justify us henceforth in using the term "slip spiral" to unify the two sets of observations, just as we have formerly proposed the term "pit spiral" to indicate the botanical relationships of this latter structure, in spite of the fortuitous and ill-defined nature of cotton hair pits. The fact that Robinson's slip-planes (spirals) are twinned, and are, moreover, not identical with the pits in timber, might appear to condemn such an attempt at unification; but we shall shortly show that such twinning and non-identity do actually exist in the primary wall of the cotton hair, and may, by analogy, well do so in wood cells also. Conversely Nodder, *loc. cit.*, has shown that the pit spirals of flax and hemp are exclusively single-handed.

In polarised light the slow spirals appear to be actual surfaces of discontinuity (fig. 18). The true relationship between them and the pit spirals will be eventually discovered by tracing both to their origin in the primary wall. As a first step in this direction we have endeavoured to determine their respective angles of inclination to the cell axis, as read at the level of the primary wall. To do this with accuracy is difficult for pit spirals, since the progressive thickening of the cell wall on a predetermined pattern would tend to produce denser packing as the cell axis is approached; and such packing

\* Robinson, W., "The Microscopical Features of Mechanical Strains in Timber and the bearing of these on the Structure of the Cell-wall in Plants," 'Phil. Trans. Roy. Soc.,' B, vol. 210, pp. 49-92 (1920).

† Notably fig. 18, bottom left-hand corner. Dr. Robinson has very kindly shown us his original prints, in which these features seem to us to be much more evident.

would produce a couple tending to intwist the spiral. It may be mentioned that our colleague, Mr. F. P. Slater, has found indications that a more rigid core of this nature does actually exist in well-thickened hairs. In agreement with this view we found that mature hairs have a pit spiral angle around  $23^{\circ}$ , and that this angle gradually increases to  $29^{\circ}$  as we examine younger hairs possessing fewer growth rings. It would seem, therefore, that the structural angle of the pit spiral, as distinguished from the modifications subsequently imposed upon it, is approximately  $29^{\circ}$ .

The determination is far more difficult with the slip spiral, since we have as yet no means for making it visible without mechanical damage and dislocation. The pit spiral angle is similarly distorted, values as low as  $17^{\circ}$  being recorded after stressing in torsion; but evidently this cannot be used as an index to provide a correction. The most we can assert at present, after comparing hairs stressed by various methods, and as gently as possible, is that the slip spiral structural angle lies (for our greenhouse Sakel in particular) in the neighbourhood of  $70^{\circ}$ .

*Distribution and Variability of Structure Reversals.*—Since the use of elliptically polarised impure light had made it actually much easier to map the structural reversals than to map convolutions (*loc. cit.*), a number of bolls which happened to have opened on one and the same plant at three-day intervals in our greenhouse were thus studied, in the hope of tracing more clearly some such day-to-day effect of environment as the convolutions had suggested.

Our intention was to map a sufficiently large number from each boll to give a typical graph of reversal distribution for that boll; but at an early stage it became evident that a flank attack on the problem would be easier. The data for the first three hairs examined from one seed only in each of the first four bolls are given in fig. 3 in order to show the difficulty of presenting the data until more is known about the desired form of presentation, as also the apparently independent individuality of every hair. It should be noted that this negative preliminary result does not contradict our earlier though still tentative observations, since we were then mapping the number of convolutions and not the number of reversals.\*

Up to this stage we had worked on the hypothesis that the reversals were predetermined upon a "pattern" laid down in the primary wall during growth in length, with a likely presumption that the point of reversal was a growth mark. The maximum number of reversals (double) was evidently much in excess of this allowance, though the modal number was round about 30, corresponding to the number of days during which growth in length continued

\* *Loc. cit.*, figs. 10-14.

in our greenhouse bolls, the mean of the full maturation period being 65 days, with secondary thickening starting on the 29th or 30th day. The number of

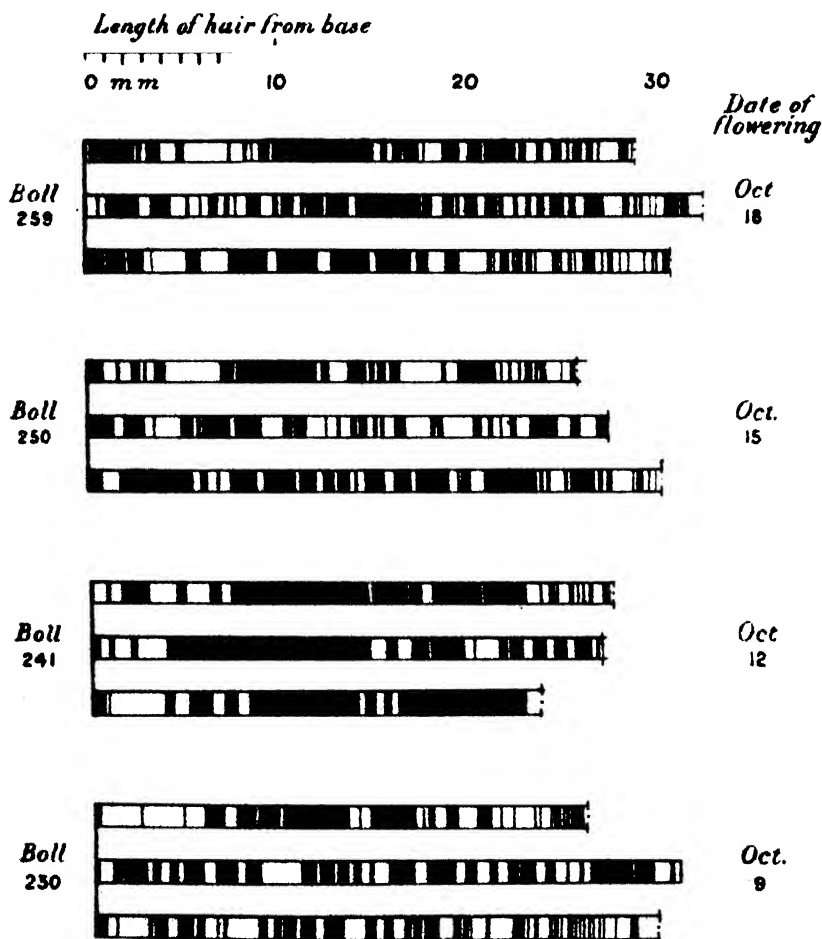


FIG. 3.—Distribution of reversals in twelve hairs.

reversals was found to be independent of the age of the boll; as soon as secondary thickening has begun, thus making reversals visible, the full and final number is present (e.g., on the 31st day). It seems, then, that if not entirely fortuitous the reversals must be predetermined.

In order to relate our greenhouse Sakel material to field-grown cotton, a few observations were made on ten hairs each of other kinds of cotton; and these gave similar values both for mean and range, while such deviations as they indicated were consistent with our scanty knowledge of the length-maturation period in other species and environments; the evidence thus

favours predetermination rather than chance. Adequately to test the hypothesis on these lines would evidently be a serious task; and we, therefore, turned again to examination of the primary wall before secondary thickening had begun. If in any way this could be made to reveal its suspected structure the difference between, *e.g.*, a 2-day and 22-day hair should be big enough to give the needed clue.

*Predetermining Structures of the Primary Wall.*—Previous experience of the phenomenon of predetermination had led up to our opinion that the primary wall did possess a structure, in spite of its fragility and its apparent lack of any patterning whatever. Various adsorption methods were devised in addition to the usual stains, and gave on examination with polarised light, feeble but definite indications of the existence of some striations in the primary wall. Priestley's discussion (*loc. cit.*) of the general problem then provided a method for converting the "pre-cellulose" into cellulose. The hair thus treated and examined with elliptically or plane polarised light presents a novel appearance. When placed with its axis parallel to the plane of polarisation or at right angles thereto, it is seen to be crossed by spiral lines, very closely set and delicate when clearly visible, but often obscured or blurred into the more obvious cloudy barring seen in the photographs (figs. 14–16). These are familiar from their resemblance to the secondary wall structures; but the new feature is that both right- and left-handed spirals occur simultaneously and equally developed in every portion of the wall. At first we attributed this to seeing through the hair to the lower side of the cylinder; but careful focussing with high powers and the cutting of longitudinal sections (fig. 17) showed that both directions of spiral co-existed in the same wall.

The next issue was the identification of these spirals by determining their angle of inclination to the cell axis. They appeared to be slow spirals, and, if so, our abandoned hypothesis (*loc. cit.*) of the slow spiral as the generator of a secondary quick spiral might be revived in a modified form. The more solid protoplasm of very young hairs made it difficult to obtain full data before the 10th day of boll development, since the protoplasm obstructed the view, but the older vesicle offered no serious obstruction when plasmolysed, and hairs from the 10th day onwards were examined in the following manner:—

The hair, after the micro-chemical treatment with KOH above mentioned, was mounted on the revolving stage, and after adjustment of the elliptically polarised illumination and suitably closing the condenser stop, was examined with the Spencer 4 mm. objective and the Zeiss 15× ocular. The latter contained a parallel-ruled graticule, and bore a pointer which moved over

a graduated circle when the graticule was rotated in order to bring its lines parallel either to the axis of the hair or to a spiral (as a double tangent to its central portion). In this way the angle made by the spiral with the hair axis was read off without any subjective error; this was

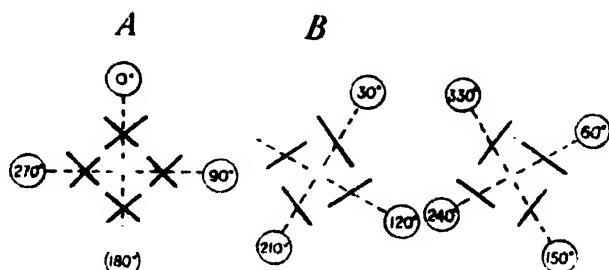


FIG. 4.—Spirals seen in primary wall under polarised light, with complete hairs variously placed with reference to the plane of polarisation.

done at first with the hair axis lying parallel to the plane of polarisation, and the hair axis was then rotated by  $30^\circ$  steps all round the circle. We thus hoped to eliminate subjective errors due to lighting, which necessarily varies as the hair is rotated, and the results are summarised in figs. 4 and 5.

These data have been given to show the nature of the phenomena as observed on the complete hair, but it is evidently preferable to make use of longitudinal sections (fig. 17). On rotating such sections through smaller steps, of  $10^\circ$ , in elliptically polarised light, we found the angles of these spirals to be inconstant, showing them to be, in part at least, merely optical effects, and not actual structures.

Thus the twinned spirals seen in four positions, corresponding to the  $48^\circ$  figured (figs. 4, A, and 5), were found to change continuously as the hair was rotated, the right-handed one rising with right-hand rotation of the hair to  $80^\circ$  right-handed after half a quadrant of rotation, beyond which a left-handed spiral marking appeared, the inclination of which fell from  $64^\circ$  to  $50^\circ$  as the quadrantal rotation was completed and the twinned spirals again appeared.

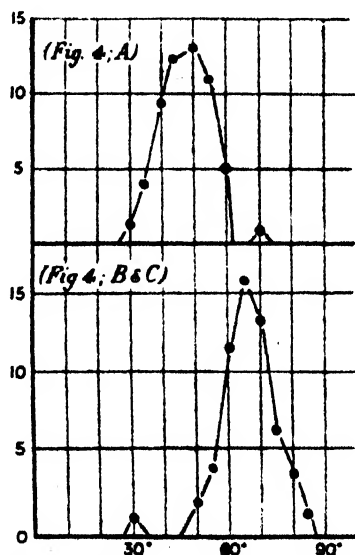


FIG. 5.—Frequency distribution of spiral angles shown in fig. 4.



We take this phenomenon to indicate that the optical axes of the structure are orientated more or less perpendicularly to the plane of the wall.

It should also be noted that both these longitudinal sections of the primary wall (fig. 17), and also the complete hairs, show only two positions of maximum extinction on such rotation between crossed Nicols. This behaviour contrasts sharply with that of the secondary wall (fig. 11), where there are four extinction positions. We are not able to state definitely that the spiral appearances of the primary wall in the 1st and 3rd quadrants of rotation are different from those in the 2nd and 4th quadrants.

An exceptional sharpness and brilliancy of colouring in some slides stained with Congo Red led to their re-examination in ordinary light, and we found that in these slides it was just possible to see and to measure an actual structure of twinned spirals without the use of polarised light. The spirals were identical in both directions; their tangential dimension was about that of the pit spiral fibril units; and their angle of inclination to the hair axis was approximately  $70^\circ$ . Attempts to photograph their delicate nexus were not successful enough to warrant process reproduction, but advantage was taken of their double refraction to intensify the photographic image by replacing the Nicol analyser in the draw tube of the microscope and setting it suitably, the polariser being omitted (fig. 17). Only one fact has been observed which gives concrete support to our belief in the existence of structural reversals in this primary wall, viz., a peculiar tendency of the longitudinal sections to tear spirally in one direction, in spite of their two-directional structure. We have not yet devised any technique for further study of this point.

In conclusion, our determinations of the structural angle of quick and slow spirals in the secondary wall on the same plants had given values of  $29^\circ$  and  $70^\circ$  *circa* respectively. It seemed justifiable to assume that one of our twinned primary wall angles is structurally identical with the slip spiral angle of  $70^\circ$  *circa* which succeeds it in the secondary wall; but this leaves the same angle in the opposite direction unaccounted for, while the  $29^\circ$  pit spiral is missing altogether in the primary wall.

*Discussion of Results.*—In the following paragraphs we shall attempt to indicate the line of interpretation which might eventually reconcile these apparently incoherent facts; but we expressly disclaim any attempt therein at an actual interpretation, since this can probably be made with far greater certainty by X-ray analysis on a progressive series of material. On the other hand, to leave these observations without any such attempt would omit their chief purpose as a biological contribution to a problem in which physics and chemistry are equally involved.

We may assume that the unit aggregate of cotton cellulose corresponds to the unit cell of the cellulose space-lattice in the Bragg sense. It seems to us very probable that cellulose is heterogeneous (thus, its "continuous reaction" under esterification may well be a statistical consequence from the co-existence of some three or four celluloses, blurred by errors of determination); if this be so, the space-lattice would be correspondingly complicated—possibly irregular or possibly compound; the unit might thereby achieve greater dimensions than in true crystals.

In the next place it is probable that whatever the dimensions of the space-lattice unit of the secondary wall may be in a tangential direction and along the fibril axis (regarding the fibril as the large structural unit), it will be small in the direction of the cell radius, since if it amounted to more than a small fraction of a growth ring ( $0.3\mu$ ) there would tend to be modal values for wall thickness according to the number of "quanta" per ring, and of such there is no present indication.

The pattern structures which we have recorded are of much greater dimensions than the largest space-lattices, but their causation is probably to be found in the space-lattice form. Now the pre-cellulose Primary wall structure is such that while itself both right- and left handed, *i.e.*, symmetrical, so far as we can yet see, it nevertheless seems able to predetermine a right- or left-handed construction of the secondary cellulose deposited upon it in any given area. It seems likely that its ostensible symmetry is not real, and that it might actually consist of two concentric layers, whether of atoms, molecules or larger units, the structures of one layer being the mirror image of those in the other layer, in which case the two layers might change position under the influence of, *e.g.*, environmental causes without affecting the appearances thus far seen by us. On the other hand, the secondary deposit at any given point, laid upon the inner layer, would develop right-handed or left-handed according to whether the one or the other layer was innermost at that point.

On such an interpretation we are almost certain to find polymerisation occurring, and the change from two extinction positions to four is also explicable by such an assumption. It would be easy to speculate in this direction, though to little purpose here, but it should be pointed out that our final determinations of these two angles were completed before it was noticed that their tangents stand very nearly in the ratio of 4:1. The conclusion seems irresistible that the pit spiral is due to polymerisation of the pre-cellulose units, while the slip spiral is the cleavage plane either of the enlarged

\* Nägeli and Schwendener, 'Das Mikroskop,' 1877 (Eng. trans.); also Pfeffer, 'Physiology of Plants' (Ewart's Transl.), p. 77 (1890).

† Herzog and Jancke, 'Zeitsch. angew. Chem.,' 1921 and 1922.

space-lattice thus formed or of an hypertrophied pseudo-lattice built up from more than one kind of cellulose.

In our previous communication we purposely refrained from quoting the pioneer work of Nägeli\* in this field, in order not to prejudice the matter by premature adherence to any school of opinion; but it now seems evident that we have almost reached an interpretation which will simply be Nägeli's micellar hypothesis expressed more rigidly and definitely than the state of physical science in his time would permit. It should be capable of being used as a starting point for the investigation of growth from the view point of the student of atomic structure. The complete interpretation will evidently be achieved by X-ray analysis, though the very imperfect results so far obtained on such definite structures, *e.g.*, by Herzog and Jancke† may possibly be due to the space-lattice units being much larger for two dimensions than in ordinary crystals and so requiring rays of longer wave-length than usual, while the spiral grouping of the units, by preventing a simple random arrangement, may also be some hindrance. The superior tensile strength of cotton as compared with other textile fibres and with regenerated cellulose filaments must depend on this construction and on the mutual chemical relations of the units of the structure. This structure may not be reproducible without the intermediation of the living protoplasm.

#### *Summary.*

In the introduction attention is called to the spongy structure of the cotton cell-wall, the specific gravity of which is nearer to 1.0 than to the value of 1.55 accepted for cotton cellulose itself.

1. Three additions to our previous methods have been utilised, *viz.*, observation in elliptically polarised light, preparation of longitudinal sections, and the development in the primary wall, by boiling with KOH, of a substance reacting to cellulose stains.

Observations have been chiefly made on hairs, at all stages of development, grown on Sakel (Egyptian) plants in a greenhouse, but checked on other material.

2. The direction of convolutions formed in isolated hairs is entirely determined by the spiral reversals of wall construction.

3. Certain chemical relationships are indicated by the following facts:—

- (a) The wall does not fall into convolutions following mere plasmolysis, but does so on drying.
- (b) This loss of constructional water is irreversible.
- (c) The structural relationships to polarised light are but little affected by strong alkalis, but are readily abolished by acids.

4. Two cases of mirror image structure appear to exist in the hair wall, though these do not necessarily imply stereo-isomerism. In both cases the surface of reversal is at a normal to the current direction of growth :—

- (a) The secondary wall visible structure is shown to form mirror-images on either side of a reversal point.
- (b) The primary wall structure is conjectured to consist of two concentric cylindrical layers (probably molecular), whose structures are mirror images. At reversal points these layers are presumed to change places.

5. The structures formerly termed by us the "slow spirals," we now propose to designate as "Slip Spirals."

- (a) The slip spirals are now shown to be invariably opposed to the pit spirals, thus resembling cleavage planes.
- (b) The single slip spiral of the cotton secondary wall is considered equivalent to the twinned slip spirals of wood cells, and it exists as a twin in the primary wall.

6. The number of structure reversals in the wall of one hair cell fluctuates round a mode in the neighbourhood of 30, indicating that a tendency to the formation of one complete (double) reversal daily during growth in length is still a possible view.

- (a) The full number is present as soon as secondary thickening begins.
- (b) No means for demonstrating the presumed reversals in the primary wall have yet been devised.

7. Two helical spirals have been found :—

- (a) One is seen in both primary and secondary wall (slip spiral) at  $70^\circ$ ; it is twinned right- and left-handed in the former only.
- (b) The other in the secondary wall, called the pit spiral, appears to have a constructional angle of  $29^\circ$ , subsequently reduced by torsion during growth in thickness.

8. The tangents of these angles happen to stand almost exactly in the ratio of 4:1, which suggests polymerisation, as does also the change in number of extinction positions.

9. Some tentative speculations as to its ultimate structure are made, in terms of a space-lattice hypothesis.

Our best acknowledgments are due to Mr. H. A. Hancock for keen and continued assistance through the work, including the making of photo-

graphs. The influence of Dr. Mary Cunningham and Mr. F. P. Slater has already been mentioned, and the greenhouse facilities have been essential. For these last we are indebted to our Executive Directors in the Fine Cotton Spinners Association, to whom we owe permission to publish these results. The routine of the greenhouse culture was undertaken by Messrs. T. Chadwick and F. Drabble. Miss M. Savill has assisted in the preparation of this account. Our thanks are due to Dr. F. F. Blackman, F.R.S., for revising the text of this communication.

A number of references have been drawn upon in the course of the research, mainly for ideas as to the direction in which it was likely to develop subsequently; of these we may note especially:—

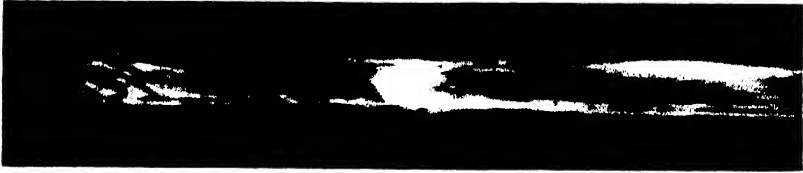
- C. F. Cross and C. Doré, 'Researches on Cellulose,' IV, etc., 1922.  
 Sir William H. Bragg, "The Structure of Organic Crystals," 'Proc. Phys. Soc.,' vol. 34, p. 33 (1921).  
 Sir William H. Bragg, "The Significance of Crystal Structure," 'Chem. Soc. Trans.,' p. 2766, etc. (1922).  
 J. J. Lynet Zwikker, "On the Constitution of the Polysaccharides," 'Rec. Trav. Chem. Pays-Bas,' vol. 41, 4th series, T. III.  
 J. C. Irvine and C. W. Souter, "The Conversion of Cellulose into Glucose," 'Chem. Soc. Trans.,' vol. 117, pp. 1489-1500 (1920).  
 R. C. Wood, 'Physical Optics' (1922).

[*Note added in Press, May 31, 1923.*—An account of independent observations ("The Structure of the Cotton Hair and its Botanical Aspects.—II. The Morphology of the Wall") published by H. J. Denham (Jour. Textile Institute, vol. 14, No. 4, April, 1923, T 85), while this account was in the press, has criticised our previous paper (Balls and Hancock, *loc. cit.*).

Denham's difficulty (p. 66) in reconciling our present "micellar" view of the structure with our former actual demonstration of growth by apposition is probably due to the influence of somewhat artificial controversies in the past, between adherents of Apposition and Intussusception. On the analogy of brick-laying, intussusception must necessarily take place, even in a single layer, if more than one operator is engaged in the work of apposition, and even with a single operator if a closed surface is being covered.

His statement (p. 66) that we invoke Church's Phyllotaxis theory to sustain our hypothesis is not correct. We expressly pointed out (p. 432) that our working hypothesis, in which we had hoped to include this theory, broke down under test, and that "our hope of confirming and extending Church's main conclusion has at present failed."

Denham's more fundamental criticisms (pp. 66-67) are invalidated by



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the fact that our structural observations are now made equally easily on untouched living hairs, and also by the definite reactions to polarised light, which could hardly be produced if the fibril structure were indiscriminate in some thirty layers, and analogous to "brush marks" running in all directions as Denham states.]

# DESCRIPTION OF PLATES 5 AND 6.

Except where otherwise stated the latter are made from Sakel (Egyptian) variety under greenhouse cultivation, immature hairs, mounted in Euparal, observed between crossed Nicols with 4 mm. Spencer dry objective, N.A., U. 66, and Zeiss 15 $\times$  ocular. Actual magnification of photographs stated.

- Fig. 6.—Complete hair; elliptical polarisation; set to uniform illumination; ordinary photographic plate. Focused to show pit spirals at a reversal point.  $\times 410$ .
- Fig. 7.—As 6, but with quartz plates set for maximum colour contrast.  $\times 410$ .
- Fig. 8.—Double reversal in another 60-day hair; plane polarisation; hair so placed as to show the dark band effect. Spiral directions recognisable; photographed on backed plate.  $\times 410$ .
- Fig. 9.—Mature convoluted Sakel hair in plane polarised light; Wratten G. and H. filters; showing one dark band.  $\times 90$ .
- Fig. 10.—Same object as 9, in elliptically polarised light, quartz set for colour contrast; six more reversal points visible.  $\times 90$ .
- Figs. 11–13.—End of a longitudinal section of hair containing a double reversal like that of fig. 8; 31-day hair (i.e., two or three-growth rings only in secondary wall) cut in paraffin; mounted in xylol.  $\times 410$ .
- Fig. 12.—Effectively as if photographed in ordinary light, on dark ground, actually in elliptically polarised, set to avoid contrast. Note position of mechanically damaged portion.
- Fig. 11.—Same object in plane polarised light (G. and H. Wratten filters) rotated to position of extinction for the pointed end and equally for the extreme opposite end. Damaged portion is in an area which is transmitting light.
- Fig. 13.—Converse of fig. 11, the hair having been rotated in the opposite direction; area containing damaged portion is extinguished.
- Figs. 14–16.—Complete young hairs (16 days) with primary walls only, no secondary thickening. Boiled 15 seconds with KOH; stained naphthamine blue, lightly washed with alcohol. Photographed in elliptically polarised light in three positions, i.e., central (fig. 15); 30° E (fig. 14); and 30° L (fig. 16) thereof, with special sensitive orthochromatic plates.  $\times 410$ .
- Fig. 17.—Longitudinal sections of 24-day hairs, cut in paraffin wax; primary wall only; developed in KOH, stained Congo red, photographed with analysing Nicol to intensify visible pattern; G. and H. Wratten filters. Sections appear are slightly oblique to demonstrate the fact that only a single wall is seen. Zeiss; water immersion and 15 ocular.  $\times 610$ .
- Fig. 18.—Fragmentary longitudinal sections of 65-day hairs cut in collodion-paraffin and showing abundant slip-spirals, developed presumably by razor shearing stress; photographed in plane polarised light on backed plate; G. and H. Wratten filters.  $\times 410$ .



*Studies in Intersexuality. I.—A Peculiar Type of Developmental Intersexuality in the Male of the Domesticated Mammals.*

By F. A. E. CREW.

(Communicated by Prof. R. C. Punnett, F.R.S. Received April 9, 1923.)

(From the Animal Breeding Research Department, University of Edinburgh.)

During the past ten years three types of intersexuality have been made the subject of detailed examination. Lillie's work on the bovine free-martin deals with a vertebrate form; that of Bridges on the fruit-fly *Drosophila melanogaster*, and of Goldschmidt on the Gipsy moth *Lymantria*, with invertebrate forms. It is the purpose of this paper to deal with a certain type of intersexuality, occurring among the domesticated mammals, which has not yet been brought into line with the general body of researches into that condition.

Abnormality of the reproductive system, taking the form of an intimate mixture of male and female structures of the accessory sexual apparatus, and associated with some degree of imperfection of the external organs of generation, is not uncommon in the domesticated animals, and many cases in the human subject have been recorded. The condition has been referred to as "pseudo-hermaphroditism" and as "tubular partial hermaphroditism," and John Hunter, in his treatment of the bovine free-martin, mentions that he had met with the same sort of abnormality in the horse, sheep, pig and goat. In the course of the last two years the present writer has examined thirty-five cases in the different domesticated mammals, and many of these have been described in detail in the 'Veterinary Journal.'

In most instances the history had been that an individual, regarded as a female during the earlier part of its life, later had assumed the secondary sexual characters and the sexual behaviour of the male. Indeed, in the goat there are cases on record in which the individual actually won prizes as an immature female, and then, later, as the time of sexual maturity approached, it became more and more male-like, its beard grew, and about the animal there hung the pungent smell so characteristic of the male. In other cases the imperfection of the external genitalia had been such that from the first the individual had been regarded as "hermaphrodite."

I wish to thank Dr. L. T. Hogben for much helpful advice and constructive criticism, Mr. M. R. V. Panikkar for his assistance in the

dissection of specimens, and Miss H. B. Fell for her help in the preparation and examination of microscopic slides.

#### DESCRIPTION OF CASES.

Altogether thirty-five cases have been examined; twenty-five of those were in the goat, seven in the pig, two in the horse, one in cattle, and one in the sheep. All the cases are very similar in the details of their anatomy, so that it is necessary to describe only certain of them minutely. The external genitalia in a few cases had the form of an unremarkable vulva and clitoris, in others the erectile organ was abnormally large though female in type, in others it was peniform but imperfectly canaliculised. In no instance was there a typical scrotum, though in several the gonads could be palpated beneath the skin of the inguinal or perineal regions. The internal genitalia in all cases consisted of paired testes, with a histological structure varying with the position of the organ, and situated somewhere along the line between the primitive position and the imperfect scrotum and a double set of structures of the accessory sexual apparatus. The relative degree of development of the structures derived from the Wolffian and Müllerian ducts respectively varied in different cases. The secondary sexual characters were imperfectly male in some cases, though definitely male in all.

In order to show the remarkable seriation displayed by these cases of intersexuality, it is now proposed to give a more detailed description of four typical cases in the pig, four in the goat, and one case in cattle.

#### *Pig No. 1 (figs. 1 and 1a).*

The animal had poorly developed tusks, and the bristles were coarser than in a female, though not as stout as those of the male. The anus was normal in appearance and situation; ventrally to it there was a tough conical structure, between which and the base of the anal orifice extended a prominent ridge of tissue; these structures occupied the position of and resembled an imperfect vulva and an abnormally large clitoris. Antero-ventrally to the conical structure was a scrotum with paired gonads; the prepuce was very small and the mammary glands weakly developed. On dissection, the following structures were identified: paired gonads and epididymes, vasa deferentia, spermatic cords, seminal vesicles, bulbo-urethral glands, prostate, urethra and abnormal penis, representing the male structures; uterus, with well developed horns and broad ligament, together with a vagina—structures of the female apparatus.

The gonads were regular in contour; the epididymis on either side fused with the termination of the uterine horn. Both horns were slightly flexuous,

with thin collapsed walls. There was no distinct differentiation between the body of the uterus, cervix, and vagina, although in the region of the neck the lumen became smaller and the internal surface was slightly marked with characteristic prominences. The vagina in fusing with urethra ended blindly beneath the prostate.

The spermatic cords were normal in structure. The vasa deferentia, taking origin in the tail of the epididymis, reached the body of the uterus and passed through the substance of the seminal vesicles to enter the urethra. Each

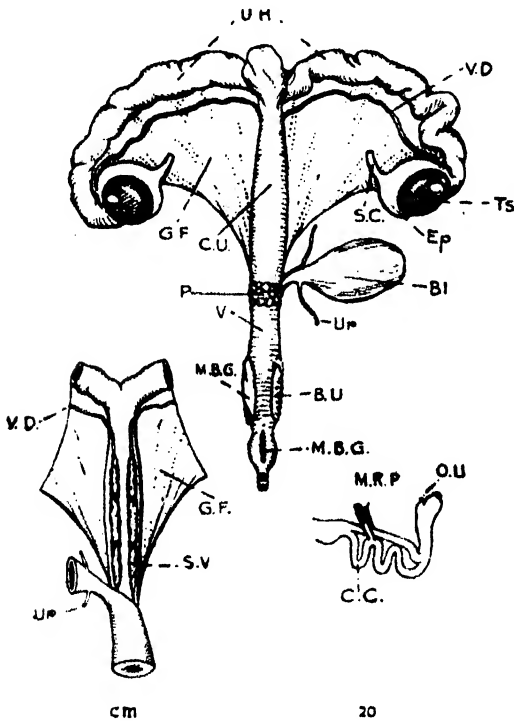


FIG. 1.



FIG. 1a.

seminal vesicle, having a dense fibrous capsule and a glandular structure, extended more than half the whole length of the utero-vaginal body to which it was attached dorsally. Ventrally the vesicles were in contact with the bladder and the ureters, but had no relation with the prostate and bulbo-urethral glands. The prostate, elliptical in shape and having the typical lobulated structure, lay obliquely across the utero-vaginal body at the place of the fusion of the vagina with the urethra; it had no relation with the seminal vesicles or with the neck of the bladder. It was composed of tissue

of pale yellow colour, denser and more compact than that of the seminal vesicles; the *pars disseminata* was not well developed.

The urethra passed from the neck of the bladder to fuse with the vagina and emerged alone from beneath the prostate, the vagina having ended blindly. In this portion its calibre was much increased and the walls thickened; the bulbo-urethral glands were small in size but normal in appearance. Further, the diameter of the urethra gradually diminished, the walls became more fibrous, and it passed along the dorsal aspect of the penis. The body of the penis was dense and compressed laterally; it took its origin near the bulbo-urethral glands and reached the perineal region, continuing backwards in a series of curves held together by connective tissue. Here it was joined by the terminal portion of the urethra and the two structures proceeded together enclosed in a covering of skin and forming the conical structure which was likened to an abnormal clitoris. The urethral part did not extend as far as the tip of the structure, and between the tip and the point where it terminated there was a narrow slit-like external urethral orifice. The ridge of tissue which extended between the anus and the base of the penis consisted entirely of a mass of striped muscle fibres, covered externally with skin.

On section the gonads were found to consist of scattered seminiferous tubules in the process of fatty degeneration. Some tubules contained a few others more numerous, germinative cells; no layer arrangement, spermatocytes and spermatids, and no sign of active spermatogenesis could be found in any tubules. Interstitial cells showed great hyperplasia; in parts, masses of these cells were separated by strands of connective tissue which was relatively small in amount. The seminal vesicles, vasa deferentia, prostate, and bulbo-urethral glands presented a normal histological picture; the uterus was normal, though undergoing fibrous degeneration.

*Pig No. 2 (fig. 2).*

The anal aperture was normal; ventral to it there was a transverse crescent-shaped opening, with a thin wrinkled dorsal lip and a ventral wall formed by a thick flattened structure with an imperforate tapering terminal portion. These structures had the form of an abnormal erectile organ and uro-genital cleft. No scrotum was present and the sex-glands could not be located by palpation.

On dissection the gonads were found to be situated intra-abdominally and to have the appearance of testes; the one on the left side was much larger in size and the surface of both was marked with greatly dilated veins. In the middle line was situated a thin-walled uterus, the cornua of which ended



*Pig No. 3 (fig. 3).*

The external genitalia were as those in the previous case, save that the structure situated ventrally to the crescentic uro-genital opening was more

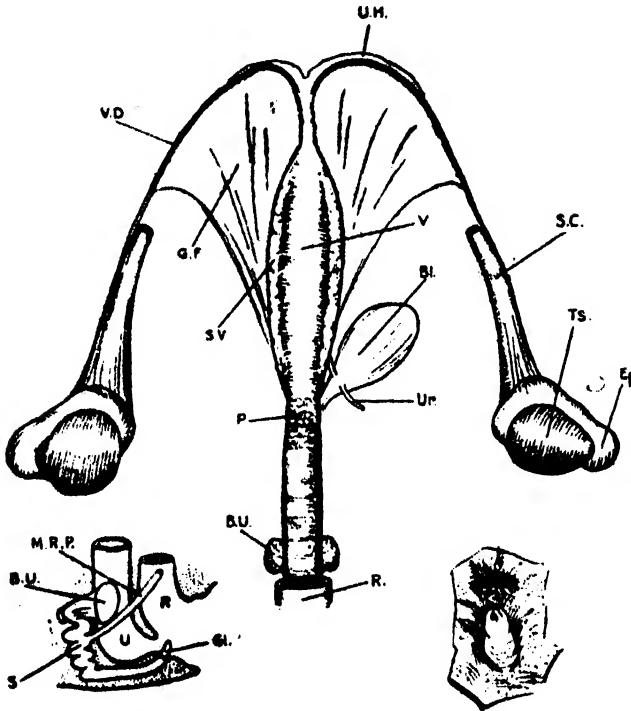


FIG. 3.

prominent and that the sex-glands were extra-abdominal though lying deep. On dissection, they were found to lie in the inguinal canal. The seminal vesicles, prostate and bulbo-urethral glands were well developed. The uterus in this case was very small and poorly developed, the cornua being short and ending blindly far from the epididymes. The thin-walled vagina had the appearance of a membranous sac attached to the prominent seminal vesicles and did not communicate with the uterus. The erectile organ was somewhat longer than that in the previous case and was situated at a higher level; it did not make such a pronounced anterior bend, but merely projected towards the external urethral orifice.

On section, the sex-glands proved to be testes with the characteristic structure of an inguinal cryptorchid.

*Pig No. 4 (fig. 4).*

The external genitalia were as those in the previous case. The internal genitalia, save for a well-marked uterus masculinus, were entirely and typically male.

*Goat No. 1 (fig. 5).*

The external genitalia consisted of a vulva-like cleft with a small non-protruding phallus in its ventral commissure. On dissection, two testes were found situated intra-abdominally, the right one being larger in size; examined



FIG. 4.

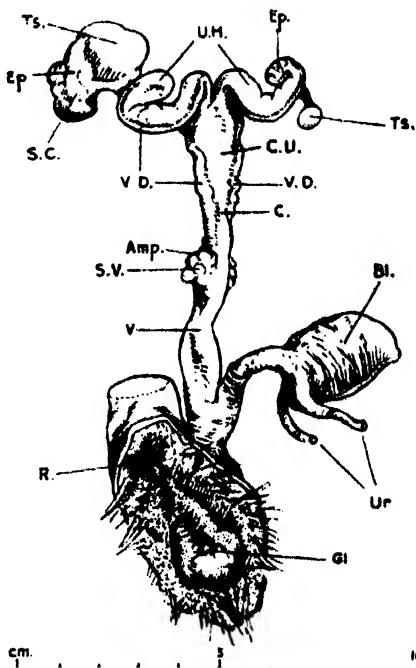


FIG. 5.

histologically, both showed the structure of abdominal cryptorchids. The right epididymis was well defined but had an unusually large head, and was not applied regularly to the testis. The one on the left was quite separate from the small testis. In the mid-line was situated the uterus. Its right horn was flexuous and ended in the region of the tail of the epididymis of that side; the left horn did likewise, but on that side the epididymis was 1.5 cm. distant from the testis. Vasa deferentia ran in the poorly developed broad ligament, to become incorporated in the ventro-lateral walls of the uterus and vagina and to pass into the substance of the seminal vesicles. As they neared the vesicles, each vas expanded to form an ampulla which was

firmly attached to the seminal vesicle. On reaching the median border of the latter each vas was joined by the duct of the vesicle to form the ejaculatory duct, which passed in the ventral wall of the vagina to open into the urethra in common with its fellow of the opposite side. The bulbo-urethral glands were extremely poorly developed, but their ducts opening into the urogenital sinus on either side of and posterior to that of the vagina could be identified. The vaginal wall fused with that of the urethra below the situation of the seminal vesicles, but their lumina remained distinct throughout their entire length. The extremely small vaginal orifice opened into the uro-genital sinus dorsally to the external urethral orifice.

*Goat No. 2 (fig. 6).*

The external genitalia were as in the previous case, but the protruding phallus was larger though similarly imperforate. The internal genitalia consisted of paired intra-abdominal testes of unequal size, the right being the

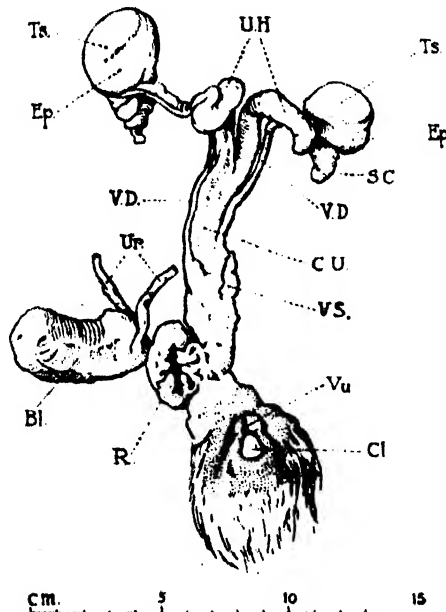


FIG. 6.

larger. The seminal vesicles were well developed. The uterus, cervix and vagina were all well defined. The bulbo-urethral glands could be identified at the point of junction of the urethra and vagina on the lateral aspect of the former. Both urethra and vagina opened independently into the uro-genital sinus.



*Goat No. 3 (fig. 7).*

In this case an ill-defined sessile scrotum was present in which one testis could be palpated. In the mid-line of the perineum 7 cm. distant from the anal orifice was the uro-genital cleft with a dorsally curved phallus. The

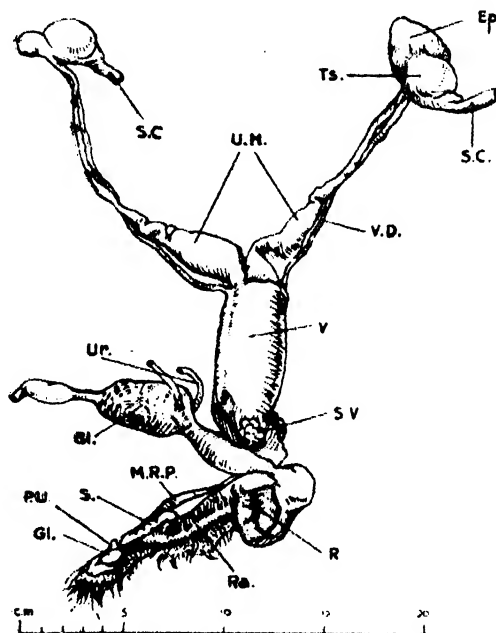


FIG. 7.

internal genitalia consisted of paired testes of equal size; the right one was situated in the inguinal canal. The epididymis on this side was cystic and contained a thin fluid in which mobile spermatozoa could be identified. The vagina was distended with a fluid content similar in appearance to that in the cystic epididymis, and communicated with the urethra by an extremely small aperture. Seminal vesicles and bulbo-urethral glands were well developed. Corpus cavernosum penis was 7 cm. in length, showed several flexures and was canaliculised.

*Goat No. 4 (fig. 8).*

As No. 3 save that both testes were situated in the ill-defined fat-containing scrotum; the epididymis was not cystic and the erectile organ was longer and presented no flexures.

*Histology of Gonads.*

The different histological pictures found in the gonads would appear to result from various degenerative changes of comparatively undifferentiated

normal testes. Such an organ was best seen in a young goat; it consisted of small tubules containing a homogeneous protoplasmic syncytium with a

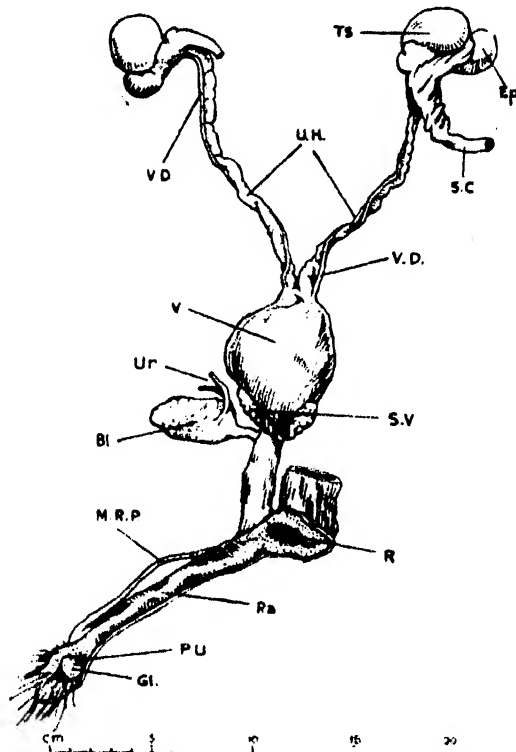


FIG. 8.

peripheral layer of small oval nuclei. No traces of spermatogenesis or mitosis were visible. The inter-tubular tissue was scanty, as in the normal animal, and consisted of small groups of Leydig cells and loose strands of connective tissue. Degenerative forms of this type could be found as follows:—

(a) Colloid degeneration of the seminal epithelium of the tubules correlated with hyperplasia of the interstitial tissue, the amount of hyperplasia being proportional to the degree of degeneration of the tubule.

(b) Fatty degeneration of the seminal epithelium and similar hyperplasia of the Leydig cells.

(c) Sclerosis, the testes being small and consisting of a few degenerate tubules of the usual type embedded in dense fibrous tissue.

(d) The testis being represented by a cyst with a thick fibrous wall enclosing the fluid. No spermatogenic tissue to be found.

In all cases where the epididymis had been examined it was found to be normal and typical.

*Bull (fig. 9).*

The external genitalia consisted of a small contracted vulva with a phallus in its ventral commissure. No scrotum was present. The internal genitalia consisted, on the right side, of an imperfect testis, with epididymis and a

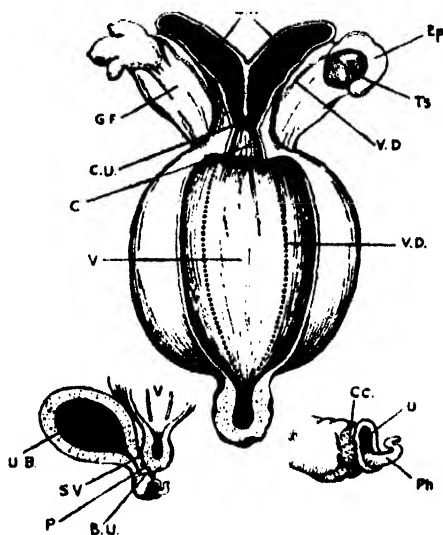


FIG. 9.

vas deferens; on the left, of a mass of fat, out of which emerged a vas deferens. In the mid-line was situated a bicornuate uterus with a cervix and poorly flexuous cornua, which terminated blindly close to the site of the gonads. The cervix was succeeded by the vagina, a closed sac distended with thick fluid content. The vasa deferentia ran parallel to the uterine horns, and, becoming incorporated in the ventral wall of the utero-vaginal body, opened into the cavity of the vagina near its blind termination. The fluid content of the vagina was examined at the Laboratory of the Royal College of Physicians and found to give reactions of *benzidin* and *tolidin* tests for blood, but no bands were seen in the spectrum; on microscopic examination the deposit was found to consist chiefly of pus cells and a few streptococci. Seminal vesicles, prostate, and bulbo-urethral glands were present, though poorly developed. The bladder appeared normal; its internal surface was covered in patches with what seemed to be inflammatory exudate; on section, the wall presented unmistakable evidence of a mild but chronic streptococcal cystitis. From the neck of

the bladder the urethra passed, surrounded by the cavernous tissue of the imperforate erectile organ, and opened into the vulva. When received for examination, the bladder, urethra and vulva were separate from the utero-vaginal body, but it was evident that when *in situ* the blind posterior wall of the vagina had been united to the dorsal wall of the urethra in the region of the prostate.

On section, both the right gonad and the tissue embedded in the mass of fat occupying the site of the left gonad proved to be highly degenerate testes. The seminiferous tubules were small and few in number, their lumina being filled mostly with desquamated cells and cell detritus. No sign of spermatogenesis, or mitosis, could be found. Intertubular tissue was present in great quantities. Numerous interstitial cells occurred in groups or singly. The seminal vesicles, prostate, and bulbo-urethral glands, though normal, showed no evidence of activity.

The uterus had an external serous layer, a thick muscular layer containing longitudinal and circular sub-layers, and a still thicker endometrium containing numerous tubular glands, which opened into the uterine cavity, and were covered with epithelium continuous with that lining the uterine inner surface. The stroma of the endometrium contained many gorged blood-vessels, and outside these were lying, in great numbers, blood corpuscles. There was no denudation of the epithelium and no streptococci were to be found in the mucosa. Vagina and cervix presented the normal structure. The uterus exhibited the histological features of that organ just previous to, or during, the phase of the oestrous cycle known as "the phase of destruction." The condition was interpreted as an infection of the utero-vagina from neighbouring parts, and was not accepted as evidence that a uterus can function in the absence of ovarian tissue.

#### *Discussion.*

The fact that all the cases readily form a series suggests that each is a grade of one and the same condition, and that between them all there is a time relation. There are but three possible interpretations of such abnormality: the abnormal individual might be a free-martin; it might be a female, not a free-martin, in which more or less complete sex-reversal had taken place; or it might be a male in which the differentiation of the sex-organisation had been abnormal.

A free-martin is a zygotic female, co-twin to a male, and the abnormality of her sex-equipment is produced by the action of the sex-hormone of the male, which passes into her body through the vascular inter-communication established in the fused embryonic membranes. For the production of

a free-martin it is necessary that there should be bi-sexual twins and fusion of the chorions. Fusion of the chorions has been demonstrated in the cow, but not in other species. Moreover, in the cases under discussion, the abnormal individuals included single births, co-twins to normal females as well as to normal males, or one of triplets, the others being normal, either males or females. Thus, though certain of these cases could be interpreted on the same principles as the bovine free-martin, all cannot be so explained.

It is known that an individual which formerly possessed the organisation of the female, and actually functioned as such, may undergo a complete transformation, and come to possess, more or less completely, the organisation of the male. But in all such cases there is the history of previous functioning, and there are certain evidences of the existence of ovarian tissue to be found. In the cases under discussion there is no history of the individual ever functioning as a female, and no evidence to show that it ever possessed only the female type of organisation.

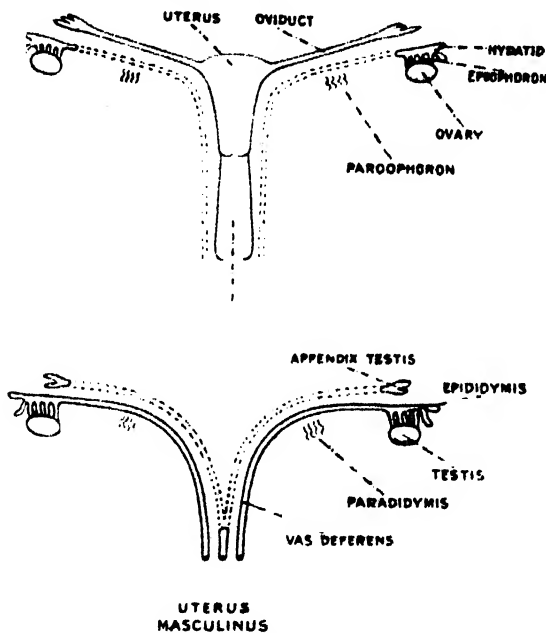


FIG. 10.

The remaining interpretation is that these individuals are males in which the differentiation of the sex-organisation has been abnormal.

Sex is determined by the nature of the factors which are brought into the zygote by the conjugating gametes and by the interaction of these within a given internal environment. Sex-differentiation is the process during which

the further development of the sex-equipment of the individual is pursued under specific control. It consists of two phases: (1) the differentiation of the embryonic gonads into testes or into ovaries; and (2) the modelling of the remaining structures of the sex-equipment under the control of the testis or of the ovary according to one of the two plans, the male or the female respectively.

At the beginning of this period of differentiation the reproductive system consists of paired gonads, a rudimentary accessory sexual apparatus composed of the growing Müllerian and Wolffian ducts, and external genitalia represented by the developing uro-genital sinus and its genital tubercle. In the male the gonads become differentiated into testes with spermatogenic and interstitial tissue, and the internal secretion elaborated by the latter, whilst encouraging the further development of the Wolffian ducts to become the epididymes, vasa deferentia and seminal vesicles, inhibits that of the Müllerian ducts, and guides the growth of the uro-genital sinus and genital tubercle into prostate, Cowper's glands, scrotum and penis.

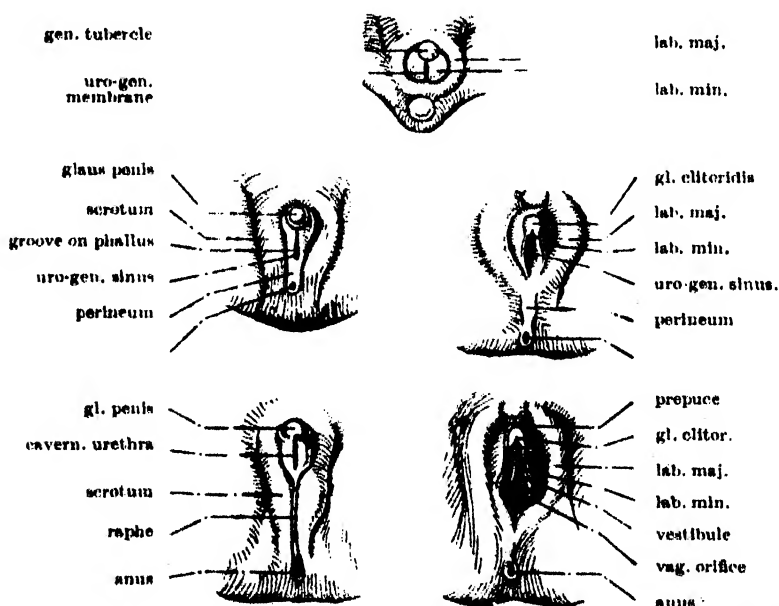


FIG. 11.—Development of external genitalia of male and female from a common plan.  
(After O. Hertwig.)

In the female the gonads become ovaries, and it is the further development of the Wolffian ducts that is inhibited and that of the Müllerian that is encouraged. The latter become fused, to form the uterus and vagina, and, becoming canaliculised, they establish a communication with the vulva,

which is developed from the uro-genital sinus. From the genital tubercle the clitoris is formed.

The time relation in the differentiation of the structures of the sex-equipment is as follows. In the earliest stage there are paired genital glands and paired solid Müllerian and Wolffian ducts. Then the genital glands become either ovaries or testes, and interstitial tissue appears, while the Müllerian and Wolffian ducts are more or less equally developed in both sexes. Next, in the female, the caudal part of the Wolffian ducts disappears, and the two Müllerian ducts fuse to form the beginnings of the uterus and vagina. In the male, on the other hand, at this stage the Müllerian ducts atrophy. In both sexes there is a common type of external genitalia. Lastly, in the female, cervix, vagina and vulval cleft are formed; in the male the scrotum and penis, whilst the testis migrates to the internal abdominal ring. The first structure of the sex-organisation to be differentiated is the gonad, the last those of the external genitalia.

Though at present experimental evidence is incomplete on this point, there are numerous clinical indications that the full development of many of the structures of the sex-organisation, or at least the rapidity with which they develop, is conditioned by the action of the internal secretion of other glands of the endocrine system, such as the pituitary, the adrenal and the thyroid. There are other sexual differences, such as those in the characters of the skeleton and in basal metabolism, which are discernible in the foetus. Differences in the male and the female pelvis, for example, are recognisable from the third month of intrauterine life. The exact relationship between the functioning of the gonad and such characters as these is not yet established.

The appropriate secondary sexual characters and sexual behaviour are exhibited later as the individual approaches sexual maturity. Since the sex-hormone is present long before this time, it would appear that a certain degree of undifferentiated growth is necessary before the threshold of response to the stimulus of the sex-hormone is reached.

The work of Lillie, Steinach, Minoura and of Sand has shown that the differentiation of the sex-organisation is pursued from the earliest stages of development under the physiological influence of the sex-gland; while that of Marshall, Steinach, Tandler and Gross, and of Sand, among others, has demonstrated that the assumption of the secondary sexual characters and the exhibition of the sexual behaviour are dominated also by the presence of a functional testis or ovary.

The fact that at one time one set of structures of the sex-equipment is affected by the functioning of the sex-gland, and at another a different set, and also the fact that the relation between the inter-sexual condition of the

accessory sexual apparatus and the degree of the imperfection of the external genitalia in the cases now being described is not constant, suggest that (1) the sex-determining substances are not produced in constant amount throughout the life cycle, and (2) there is a different threshold of response to the sex-differentiating stimulus at different stages in the development of any organ which is susceptible to such a stimulus.

It is known that in the adult female mammal vestiges of the Wolffian ducts and of the structures developed therefrom are commonly found, and that in the adult male there are to be seen the remains or the derivatives of the Müllerian ducts. The degree of the development of these structures varies widely in different cases. It follows, therefore, that either the time of the exhibition of the stimulus that controls the differentiation of the sex-organisation or else the potency of the stimulus is variable.

In the case of the inter-sexual individuals of *Lymantria*, Goldschmidt encountered a similar sort of seriation as is described here and comments upon it as follows:—"If we now try to formulate a rule which governs this strange seriation we find the most important fact, that this series is the inverse of the order of differentiation of the organs in development. The last organs to differentiate in the pupa and the first to become inter-sexual are the branching of the antennæ and the coloration of the wings. The first imaginal organ differentiated and the last in the series to be changed towards the other sex is the sex-gland. And if we apply this law to the minute parts of a single organ we find it to hold here also." The intersex in *Lymantria* is a sex-mosaic in time.

In the case of the intersexual mammal now being discussed it is possible to apply a somewhat similar interpretation of the seriation of events if it is recognised that the stimulus to differentiation of the sex-equipment becomes localised in the sex-gland; that the abnormalities pertain only to the earlier stages of sexual development; that the influence of the gonad in the mammal at this stage is such as inhibits the further development of the accessory sexual structures of the alternate sex, which would develop unchecked in the absence of such inhibition; and that in all probability there exists a different threshold of response to the sex-differentiating stimulus in the case of different structures of the sex-equipment, and at different times during the development of one and the same structure.

The accompanying text-figure gives a speculative interpretation of the phenomena along the lines suggested by Goldschmidt. The hypothetical amount of the sex-differenting stimulus is plotted along the ordinate and the time of differentiation along the abscissa. There is undoubtedly an orderly sequence in the differentiation of the different structures of the sex-equipment



in a zygotic male, and this can be shown for purely illustrative purposes as consisting of three somewhat overlapping periods: (1) The atrophy of the

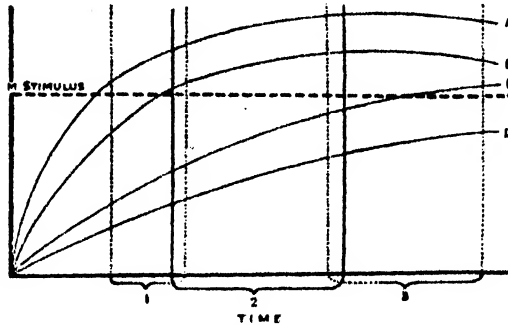


FIG. 12.

Müllerian ducts, (2) the further development of the accessory sexual apparatus, and (3) the modelling of the external genitalia. For the sake of simplicity it is assumed that for all these structures concerned there is one and the same minimum stimulus which, provided by the sex-differentiating substance elaborated by the testis, will evoke the specific response toward appropriate development. It is also assumed that when once the undirected development of any structure has proceeded so far, then that structure is no longer capable of responding to the stimulus provided by the testis.

In A the minimum stimulus necessary for proper differentiation of the sex-organisation was exhibited before the time for differentiation had been reached, and as a consequence the differentiation would be such that a completely male organisation would be established. In B in consequence of a retardation in the elaboration of the sex-hormone or of the production thereof at a slower rate the growth of the Müllerian ducts would be partially unchecked and the end result of sex-differentiation would be a male in which a *uterus masculinus* would be present. In C the end result would be a male with abdominal testes, a double set of structures of the accessory sexual apparatus, and external genitalia of the male type but imperfect. In D in which the required stimulus was never exhibited there would be no differentiation.

In the absence of the proper endocrine control during the period of differentiation Wolffian and Müllerian ducts pursue an equal and parallel development under the common stimulus of nutriment, and the uro-genital sinus with its genital tubercle increases in size to form a large cleft with a phallus in its ventral commissure. The fact that in these circumstances epididymes, vasa deferentia, and seminal vesicles are developed from the Wolffian ducts, and uterus and vagina from the Müllerian, shows that the development of these structures is not conditioned by the endocrine control

at all, and that the action of the sex-differentiating stimulus is limited to the inhibition of the development of one and the encouragement of that of the other of these paired ducts.

If such undirected growth continues, then, after a time, the structures concerned will have lost all embryonic plasticity, and even though the proper endocrine stimulus then be exhibited, they will no longer be able to respond. The degree of the development of the structures derived from the Müllerian ducts found in a male, and the degree of imperfection of the external genitalia will provide, therefore, some indication as to the time during development at which the sex-hormone became operative.

Moreover, if it is assumed that the testis, and the structures concerned in its migration, pursue a corresponding and parallel development up to the point at which descent normally occurs, and that if the proper development of the testis is retarded in any way, this association of testis and gubernacular apparatus is prevented, then mal-descent of the testis can be interpreted in terms of abnormal differentiation of the sex-organisation.

There is then an embryonic but full-grown form of the sex-equipment, and, in the cases being described, the Wolffian and Müllerian derivatives attain a considerable size, and the external genitalia have the form, in the higher grades of the inter-sexual condition, of a uro-genital cleft and a phallus. In such a case the individual, so far as external sexual characters are concerned, will resemble a female very closely indeed, and will be regarded as a female during the earlier part of its life.

The primary cause of the abnormality might be: (1) A complete absence of the interstitial tissue in the embryonic testis, or a complete non-functioning of this tissue during the period of differentiation of the sex-organisation; (2) a quantitative, or qualitative, insufficiency of the sex-hormone during this period; or (3) a mal-development, or a mal-functioning of the other endocrine glands, the action of which, in addition to that of the sex-gland, is necessary for the proper development of the structures of the sex-equipment during this period.

It has not been possible, as yet, to define the exact cause, for only well-grown individuals have been available for examination, and in these nothing remarkable in the thyroid, pituitary, adrenal, and other glands of the endocrine system has been found.

Whatever may prove to be the exact primary cause, the condition can certainly be interpreted as the result of the absence during the period of sex-differentiation of the proper endocrine control in a zygotic male, and in view of Goldschmidt's work it is reasonable to regard it as being due to an insufficiency of the sex—differentiating stimulus.

Granting this, and assuming that the threshold of response on the part of the structures of the secondary sexual characters is lower than that of the structures of the accessory sexual apparatus, it can be seen that, as the individual approaches sexual maturity, it will assume the secondary sexual characters of the male, and exhibit the male behaviour. The structures concerned in the development of these characters are the only ones of the sex-equipment which are capable of responding, and in most cases the secondary sexual characters of the male are but the result of the further development, at a certain time, of structures possessed in common by the embryonic full-grown form, the female, and by the immature male. The normal exhibition of the secondary sexual characters by these individuals can be explained equally well, on the assumption that the primary cause of the inter-sexual condition is not an insufficiency of the sex-differentiating stimulus but a retardation in its exhibition.

The frequency of the occurrence of this type of abnormality in certain strains of goats and pigs, and the fact that it is more common among the offspring of certain individuals than among those of others, suggest that as in the case of *Lymantria*, the condition is genetical in origin. In the goat there is considerable evidence to show that this kind of abnormality is related to the importation, in 1897, of three Toggenburg she-goats in kid. These goats figure largely in the back part of the pedigree of most modern British goats, according to Davies, and it is reasonable, therefore, to hold that this peculiar inter-sexual condition is the result of the mating of two races, or individuals, which differ one from the other in the nature of the factors which play their part in the determination of sex, and in the mechanism which controls the rate of sexual development.

#### KEY TO LETTERING ON TEXT-FIGURES.

<i>Amp.</i> .....	ampulla of vas deferens.	<i>P.U.</i> .....	processus urethræ.
<i>Bl.</i> .....	urinary bladder.	<i>R.</i> .....	rectum.
<i>B.U.</i> .....	bulbo-urethral gland.	<i>Ra.</i> .....	raphe.
<i>C.</i> .....	cervix.	<i>S.</i> .....	curves of corpus cavernosum.
<i>C.C.</i> .....	corpus cavernosum.	<i>S.C.</i> .....	spermatic cord.
<i>C.U.</i> .....	corpus uteri.	<i>S.V.</i> .....	seminal vesicle.
<i>Ep.</i> .....	epididymia.	<i>Te.</i> .....	testis.
<i>Gl.</i> .....	glans of erectile organ.	<i>U.</i> .....	uro-genital cleft.
<i>G.F.</i> .....	genital fold.	<i>U.B.</i> .....	urinary bladder.
<i>M.B.G.</i> ...	bulbo-glandularis muscle.	<i>U.H.</i> .....	uterine horn.
<i>M.R.P.</i> ...	retractor penis muscle.	<i>Ur.</i> .....	ureter.
<i>O.</i> .....	external urethral orifice.	<i>V.</i> .....	vagina.
<i>P.</i> .....	prostate.	<i>V.D.</i> .....	vas deferens.
<i>Ph.</i> .....	phallus.		

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## *The Composition of the Cell-Wall at the Apical Meristem of Stem and Root.*

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# INTRODUCTION.

It is a striking feature of the growth of any highly organised plant body that the construction of new protoplasm and consequent formation of new cells is usually strictly localised to certain definite regions, known generally as the meristematic tissues. In the normal flowering plant, the main meristematic regions of the axis are found at the apices of stem and root as the apical or polar meristems and distributed in the intercalary region as two thin cylinders of cambial meristem, one between xylem and phloem, the vascular cambium, the other the cork phellogen, situated near the periphery.

No tissues are more important in plant development than these meristematic regions, but so far their study has mainly been carried out by cytological methods, which have supplied much information as to the structural organisation of the protoplast, and especially of the nucleus. In the present paper, two of these meristematic regions, namely, the polar meristems of shoots and roots, are studied with reference only to the biochemical changes that proceed within the wall separating the protoplasts.

Originally these walls are extremely thin, and from general considerations, as well as from cytological observations upon the phenomena at the completion of anaphase, it would appear that these walls, commencing as interfaces in a protein-containing medium, may be regarded as composed at first mainly of protein. The original wall may be homogeneous in physical structure, but will be of extremely complex chemical nature. From the observations that follow it would appear that its subsequent history represents chemically a progressive simplification; the constituent substances segregate into special lamellae as they are released, so that the change is accompanied by an increasing complexity of organisation, of which the distinction between middle lamella and inner wall is the first visible indication.

In the literature of plant micro-chemistry there are many scattered statements as to the nature and reaction of the walls of the meristematic regions; it has been our aim to correlate and extend these observations, so as to enable a detailed comparison to be made between the walls of the two types of polar meristem. Differences of behaviour in microchemical reaction are difficult to interpret, and whenever practicable the explanations advanced have been checked by macrochemical manipulation. This latter method involves the slow and laborious accumulations of material, and these first experiments are consequently still far from quantitative, although every effort has been made to make the macrochemical observations strictly comparable for the two main types of meristem under consideration. It was thought advisable to limit these macrochemical observations to one species, and although the microchemical observations they are designed to interpret are of very general occurrence amongst flowering plants, the macrochemical data refer only to the shoot and root of *Vicia faba*, L., of which two varieties were used, "Windsor" and "Prolific Long-pod," supplied by Messrs. Sutton and Sons, Reading. Some very similar experiments have been carried out with the Scarlet Runner bean, *Phaseolus multiflorus*, Willd., but they are only referred to because they have suggested that possibly the broad-bean may be exceptional in the very large amount of alkali soluble protein contained in the ungerminated radicles.

It was pointed out in an earlier paper (20), that owing to the relative

impermeability of the protoplasts of the meristem and their need for a constant supply of organic solutes, if the active synthesis of protoplasm essential to growth is to be maintained, the walls separating the protoplasts become of very considerable importance, as they seem to be the natural channels by which the distribution of these solutes must take place in the meristem. Certain reasons were given in the earlier paper for the conclusion that some of the differences in the manner of growth of roots as compared with shoots, might be traced in part to the slower diffusion of such solutes in the meristem of the root than of the shoot. It is therefore of great interest that the present biochemical study reveals marked and essential differences between the walls of the two polar meristems. In the root meristem the walls contain a much greater proportion of protein and fatty acid than in the stem meristem, where cellulose and pectic substance preponderate; the presence of fatty acid and protein would undoubtedly impede the diffusion of water-soluble solutes through the meristem wall.

In the course of this work the closer analysis of the meristem wall has made it necessary to distinguish five types of apical meristem. Root meristems are practically very much alike, but it is advisable to distinguish the meristem of (i) the ungerminated radicle from that of (ii) the growing root, whilst in the shoot essential differences have revealed themselves between (iii) the ungerminated plumule, (iv) the shoot apex grown in darkness, and (v) the shoot apex grown in light.

#### I. MICROCHEMICAL REACTIONS OF THE APICAL MERISTEM.

The characteristic microchemical reaction of a differentiated tissue system, such as is found just behind the apical meristem of either stem or root, is the readiness with which the walls of the general ground tissue turn blue when treated with iodine in aqueous potassium iodide after hydrolysis with a suitable reagent. This is the characteristic cellulose reaction, and will be given by all tissues except specially differentiated xylem elements, in which the lignified wall reacts but slowly, and fat impregnated protective layers (the *Schüttscheide* of the German writers), such as a superficial epidermis or deeper seated endodermis. As the tissues grow older, the cellulose itself frequently becomes more resistant to hydrolysis, the walls are incrustated or impregnated with more resistant substances so that the cellulose reaction is less readily given, but the starting point of the present investigation was the observation that adult differentiated tissues give characteristic reactions, both for cellulose and pectin, far more readily than any meristematic tissue, except, perhaps, that of the normal shoot growing in the light.

While the meristem walls of radicle, plumule and root are unchanged in appearance by treatment with iodine and sulphuric acid, those of the green and etiolated stem apices swell up and turn blue, the swelling being less marked in the etiolated than in the green stem. (It is important that only pure sulphuric acid be used, and reliable results are obtained using it at a strength of 70 per cent. Sulphuric acid frequently contains impurities capable of acting as oxidising catalysts, and the blue reaction is then immediately obtained. This may be associated with the fact that, after treatment with such an oxidising agent as Eau de Javelle, *i.e.*, alkaline potassium hypochlorite (Molisch (15)), the blue reaction is given with iodine and pure sulphuric acid.)

The meristem of radicle, plumule and root of *Vicia Faba* give, however, the cellulose reactions with iodine and 70 per cent. pure sulphuric acid after any of the following alternative treatments:—

(1) Sections soaked in ammonia (sp. gr. 0.88) 48 hours, followed by Eau de Javelle for 24 hours.

(2) Sections soaked in Eau de Javelle for two to three days.

(3) Sections boiled with 10 per cent. aqueous caustic potash or caustic soda for a few minutes.

(4) Sections boiled in 10 per cent. sulphuric acid for a few minutes.

(5) Sections boiled in 10 per cent. hydrochloric acid for a few minutes.

But still no reaction for cellulose is given with chloriodide of zinc, which throughout the work was used as two separate solutions, zinc chloride and iodine in aqueous potassium iodide, as suggested by Artschwager (1). Uniformity in the preparation and employment of this reagent is very essential if reliable results are to be obtained.

Further experiment showed, that to obtain the cellulose reaction with chloriodide of zinc at the meristem of root, radicle and plumule, one of two alternative preliminary treatments is necessary; the sections must either be (1) boiled for a long period in strong (40 per cent.) aqueous potash or soda, or (2) boiled for a short time in alcoholic potash.

The walls of the meristem of the etiolated stem differ from the above types only in giving the cellulose reaction with iodine and sulphuric acid without previous treatment, and in the relatively shorter preliminary treatment with aqueous or alcoholic alkalis required to produce the reaction with chloriodide of zinc.

The meristem walls of the green stem, on the other hand, though they do not give the cellulose reaction with chloriodide of zinc direct, give it with much greater readiness, *viz.*, after any of the following treatments:—

- (1) Sections 1 hour in Eau de Javelle (cold).
- (2) Sections boiled in 25 per cent. hydrochloric acid in alcohol.
- (3) Sections boiled in 5 per cent. aqueous hydrochloric acid.
- (4) Sections boiled in dilute (2 per cent.) aqueous potash or soda.
- (5) Sections boiled in alcoholic potash (an intense cellulose reaction subsequently).
- (6) Sections soaked in cold concentrated potash (an intense reaction subsequently).
- (7) Sections soaked in cold concentrated aqueous hydrochloric acid (slight reaction subsequently).

We see, then, that the reactions of the various meristems towards cellulose reagents places them in three distinct categories, viz. :—

- (1) That of radicle, plumule and root.
- (2) That of etiolated stem.
- (3) That of normal stem growing in light.

The differences between these types of meristem will be analysed in a series of discussions of the presence and distribution of various substances found to play an important part in the construction of the meristem wall, viz., celluloses, protein and pectic substances, and fatty substances and their salts, notably the calcium soaps.

## II. CELLULOSE AND PROTEIN IN THE MERISTEM WALL.

### A. *Cellulose.*

It has been shown in an earlier paper (20), that while a normal cellulose wall dissolves relatively easily in concentrated pure sulphuric acid, the walls at the meristem of the root are comparatively resistant and dissolve only slowly, without giving the characteristic cellulose reaction with iodine reagents at any stage. The normal oxycellulose or decomposition product of cellulose, which stains with iodine, is not recognisable as the wall slowly breaks down and dissolves in the concentrated acid. The walls at the meristem of plumule and radicle have now been found to behave in this respect as those of the root. But the fact that in each case, after short treatment with boiling alkali, these walls dissolve readily, giving the blue reaction with iodine reagents, suggests that cellulose, or a precursor of cellulose, is present in the meristem wall; though probably so combined into a complex molecule, that as the molecule is slowly broken down by sulphuric acid, the cellulose is destroyed before it is freed from its associated linkages and while it is unable to show the characteristic reaction.

The following macrochemical experiment proves clearly that cellulose is



liberated from the walls of the radicle, only as the result of previous treatment with alkali. 16 grm. of dry radicles were separated from dry broad-beans, ground into a fine powder, and extracted for 48 hours with Schweizer's solution (cupric hydrate dissolved in 0.88 ammonia (Haas and Hill (10)). The extract was filtered through Kahlbaum asbestos on a Buchner funnel, and the clear blue solution acidified with concentrated hydrochloric acid. The dense-flocculent precipitate obtained was washed several times in water by decantation, and then collected on a filter paper in a Buchner funnel.

This precipitate slightly greyish-white in colour, tested with chloriodide of zinc, remained unchanged except for very occasional specks here and there which gave a strong blue reaction. The precipitate dissolved almost completely in ammonia, or in caustic soda and potash, the few white specks remaining undissolved all gave the cellulose reaction with chloriodide of zinc. The alkali-soluble precipitate gave magnificent reactions for protein, especially the tyrosin reaction (Millons and the xanthoproteic), a strong biuret reaction, very slight cystine reaction with lead acetate and caustic soda, and a very slight Molisch reaction for carbohydrate (see Plimmer (22)).

These results are compatible with the assumption that Schweizer's solution dissolves out a large bulk of globulin-like protein, soluble in concentrated ammonia as well as in dilute aqueous alkali, together with the small traces of cellulose always present in the root cap and at the base of the radicle, where it joins the hypocotyl.

In view of the complete solubility in aqueous alkali and the slight reaction with  $\alpha$ -naphthol and sulphuric acid, there seemed little likelihood of the presence of any cellulose complex in the precipitate. But as a considerable amount of the precipitate was available, it was dissolved slowly and with as little warming as possible, in 75 per cent. sulphuric acid, and then diluted down to 10 per cent. with distilled water. This solution was boiled for about 10 hours on a water-bath under a reflux condenser. A black and sandy precipitate separated out at an early stage during the process, and was obtained on every occasion when this hydrolysis was repeated. The solution was filtered, neutralised with solid calcium carbonate and the neutral solution sucked away from the paste on a Buchner funnel. When the solution was evaporated to dryness, no syrup was obtained, but only a slight organic residue which was soluble in alcohol, crystallising from alcoholic solution in clusters of very small needles and *laevo*-rotatory in alcoholic solution. The concentrated aqueous extract did not reduce Fehling's solution and gave no osazone on warming with phenyl hydrazine hydrochloride, sodium acetate and a drop of acetic acid. The precipitate

from the Schweizer extract of the bean radicle is therefore probably mainly protein in nature and contains no cellulose complex.

The residue from the radicles, after extraction with Schweizer's solution, was collected from the asbestos on the Buchner funnel and boiled for an hour with 100 c.c. of 10 per cent. sodium hydrate. It was then filtered through asbestos, washed with water and the swollen slimy residue again extracted with 500 c.c. of Schweizer's solution. A copious precipitate was again obtained on acidifying with hydrochloric acid, and in this case gave a strong cellulose reaction with chloriodide of zinc. The precipitate was hydrolysed in 10 per cent. sulphuric acid. No precipitate formed; during hydrolysis the solution remained clear and pale yellow in colour. After neutralisation with calcium carbonate, filtration and evaporation of the neutralised solution, a few cubic centimetres of a syrupy liquid were obtained, which reduced Fehling's solution and gave the characteristic dextroazone crystals in the hot solution. The aqueous liquid had a well-marked dextro-rotatory action on polarised light and there is little doubt that *D*-glucose was present, formed by the hydrolysis of cellulose present in the residue from the radicles after boiling with alkali.

To prove the protein nature of the substance first extracted by Schweizer's solution, 20 gm. of ground-up radicles were extracted for 48 hours, the solution filtered through Kahlbaum asbestos, and the filtrate acidified with concentrated hydrochloric acid. The precipitate was washed several times by decantation, then redissolved in 10 per cent. potassium hydrate to avoid the possible formation by the protein of an ammonium salt. The protein was reprecipitated from solution with hydrochloric acid, filtered, washed and dried in a vacuum desiccator. The total nitrogen, as estimated by Kjeldahl's method, was 15.4 per cent. The protein extractable from 10 gm. of broad bean plumules by Schweizer's solution was similarly treated, and yielded 16.5 per cent. nitrogen on estimation by a Kjeldahl. These figures leave little doubt that an alkali soluble protein is present in the meristems, which can be in part extracted by Schweizer's solution, whilst this cellulose solvent fails to remove practically any cellulose unless these meristems are previously boiled with alkali, a treatment which extracts the greater part of this protein. In the next section grounds are given for thinking that a protein of this type is actually an important compound of the meristem wall in root, radicle, and plumule.

#### *B. Protein in the Meristem Wall.*

A consideration of the micro-chemical reactions on p. 114, shows that the substance, which in combination with cellulose renders the latter so resistant

to sulphuric acid and prevents the formation of the usual decomposition products, can be removed from the meristem wall of root, radicle and plumule by certain reagents. After this treatment, the wall, when dissolved in sulphuric acid in the presence of iodine reagents, gives the blue colour characteristic of cellulose.

The fact that even then the cellulose reaction is not given with chloriodide of zinc, suggests that yet another substance is linked with the cellulose, which is removed by prolonged treatment with caustic alkalis, and is shown in a later section to be in all probability a fatty acid.

Mangin (13) has already suggested, that in the young cell-wall, as in the adult parenchymatous wall, cellulose exists in a state of combination with a pectic substance, but it is difficult to believe that this pectic complex can be responsible for the resistance to sulphuric acid, for the following reasons:—

(1) The ready hydrolysis of all pectic substances in strong sulphuric acid.

(2) The fact that meristem walls of the etiolated stem, which give the cellulose reaction immediately with iodine and sulphuric acid, contain if anything more pectin still more firmly combined.

(3) That the meristem walls do not stain with such a pectin stain as methylene blue, even after treatment with alcoholic hydrochloric acid, while the differentiated walls stain intensely.

On the whole, a combination of protein with the cellulose, seems the most probable explanation of this resistance to sulphuric acid. Such a protein-cellulose complex derives some support from the secondary considerations suggested by the chemical nature of the basal substance of the Casparian strip (Priestley and North (19)).

During an experiment to be fully described later (p. 123), 20 grm. of radicles, finely ground, after 72 hours' extraction with cold Eau de Javelle and the removal of all fatty substances by various means, were boiled for three-quarters of an hour with 5 per cent. aqueous hydrochloric acid. The solution filtered hot, became opalescent as it cooled; when neutralised with ammonia a precipitate formed which gave all the usual protein reactions (Plimmer (22) *loc. cit.*, p. 365). The filtrate, after removal of the protein, poured into absolute alcohol, gave a copious precipitate of pectin.

This suggests how very intimately some of the protein constituents seem to be linked with the meristem wall. None of these considerations are conclusive, but they lead to the tentative suggestion that the meristem walls of the radicle and probably of the root and plumule also, owe their resistance to strong sulphuric acid and the failure to give the cellulose reaction with iodine and sulphuric acid, to the fact that cellulose, or a related carbohydrate, is present in complex union with a protein.

That this protein is not the protein extracted by Schweizer's solution or concentrated ammonia, is clear from the observation that sections of radicle and plumule left 24 hours in either Schweizer's solution or ammonia, then washed and treated with iodine and strong sulphuric acid, do not give the cellulose reaction in their walls.

Some experiments in which equal weights of ground up dry radicles and plumules were extracted successively with: (1) Eau de Javelle, cold, 72 hours, (2) 0.5 per cent. ammonium oxalate on a boiling water bath for half-an-hour, (3) boiling 5 per aqueous hydrochloric acid, do not deserve description in detail but are noteworthy because they showed clearly that after the prolonged preliminary treatment with Eau de Javelle, the walls of the meristem of the radicle yielded in the successive extractions considerably larger quantities of protein than the walls of the plumule.

Microscopic control of material treated in this manner with Eau de Javelle makes it absolutely certain that the protein obtained in subsequent manipulations cannot arise from the cell contents. Tissue treated with this strong alkaline oxidising agent for such long periods, shows the cells completely cleared of all contents, so that nothing but a net-work of wall remains to undergo further experimental treatment.

### III.—PECTIC SUBSTANCES IN THE MERISTEM WALL.

As these substances, in part anhydrides of pentose sugars, are not too well characterised, the group may be treated as including the following categories:—

(1) *Pectin* (= pectose of many authors—pectinogen of Schryver and Haynes (23)) forms a colloidal solution in water, very soluble in dilute ammonium oxalate, precipitated from solution by alcohol, or in presence of calcium precipitated as calcium pectate—in presence of cold dilute alkalis or of pectase, methyl alcohol and acetone are eliminated from the molecule (Tutin (27)).

(2) *Pectic acid* only very slightly soluble in water giving a colloidal solution, but the sodium and potassium salts very soluble, the calcium salt insoluble.

(3) Products of hydrolysis, more acid in nature, such as metapectic acid, probably identical with arabic acid, going into true solution in water.

Details as to chemical behaviour, microchemical reactions, etc., will be found in such monographs as Tollens (26) or in text-books such as Ouslow (16) and Haas and Hill (10). Mangin (13) has shown that pectic substances are present in the meristem wall. The present problem is the explanation of differences in reaction towards pectic stains of the various meristems and the relative ease of extraction of the pectic substances during microchemical

investigation. It is here that differences first appear between the meristem of radicle and plumule.

Radicles and plumules were isolated from the broad-bean by breaking open the dry testa and pressing apart the cotyledons, then separating the two small dry structures with a knife. Although these structures were separated from many kilograms of dry seed, as their individual weights are of the order of plumule 3 to 4 mgrm., radicle 7 to 8 mgrm., the quantities available have set a limit to the macrochemical scale of operations throughout the investigation.

Equal weights of dry radicles and plumules from the broad-bean were ground up and extracted with a warm aqueous 0.5 per cent. solution of ammonium oxalate for half-an-hour.

[*Note*.—This type of comparative experiment occurs frequently. In each case equal weights of original substance were treated throughout in an identical manner with equal quantities of the same reagents, all apparatus used being in duplicate, so that roughly quantitative comparisons could be made.]

The solution was then filtered off through a cloth and absolute alcohol added to the filtrate; about equal flocculent precipitates were obtained. Tested for protein with xanthoproteic, Millon and biuret reaction (Plimmer (22), *loc. cit.*, p. 365, the same technique used wherever these reactions are quoted), the precipitate from the radicles gave better tyrosin reaction, but both precipitates gave good biuret reactions. The precipitates were then dissolved in dilute alkali, allowed to stand for 10–15 minutes and then tested for pectin with the phloroglucin and orcinol reaction (Onslow (16), *loc. cit.*, p. 44), which gave positive results in both cases.

The radicles and plumules were then boiled for half-an-hour with 5 per cent. aqueous hydrochloric acid, filtered, and the pectin precipitated with alcohol. These gave in each case a slight biuret reaction, good xanthoproteic and Millons reaction, not quite so good from the plumules. The pectin reactions were as follows:—

Radicles.	Plumules.
Phloroglucin, slight reaction.	Slight reaction.
Orcinol-Ferric chloride, no reaction.	Good reaction.

It would seem from the above experiment that the pectic substances whether from the wall or the protoplast, are more readily removed from the radicles than from the plumule.

The following results suggest, that in addition to these pectic substances, a pectic complex is present in the wall of the radicle, but is not so firmly attached as the pectic complex in the plumule.

One gram. each of radicles and plumules was ground up and extracted for

17 hours with ammonia (sp. gr. 0.88), filtered, washed with distilled water, and the filtrates acidified with hydrochloric acid. A flocculent precipitate formed in each case, but greater from the radicles, to judge from the rate of settlement in an Esbach tube; the precipitates were in bulk as 3:2. Examined precipitates with the following results:—

Radicles.	Plumules.
Xanthoproteic, good reaction.	Good reaction.
Millons, good reaction.	Good reaction.
Biuret, slight reaction.	Slight reaction.
Molisch, no reaction.	No reaction.
Furfural,* good reaction.	Good reaction.
Phloroglucin, no reaction.	No reaction.
Orcinol, no reaction.	No reaction.

The acidified filtrates on neutralisation with lime water gave further precipitates which gave protein reactions.

The radicles and plumules were then extracted for 72 hours with Eau de Javelle, filtered and washed with distilled water. Absolute alcohol added to the filtrate gave a slight precipitate, which gave no protein reaction but a good furfural reaction in both cases, and from the *plumules only* a good orcinol and phloroglucin reaction. Presumably the long exposure to Eau de Javelle, after concentrated ammonia, had so well oxidised and dissolved the proteins that the precipitate thrown down by alcohol in neither case shows a protein reaction.

The residues of the radicles and plumules were again extracted for half-an-hour on a water-bath with 0.5 per cent. ammonium oxalate, filtered and absolute alcohol added to the filtrates. Both gave precipitates of pectin only but nearly twice as much from the radicles. The residues were then left for three-quarters of an hour in cold 25 per cent. hydrochloric acid, filtered, washed with alcohol and warmed for half-an-hour on the water-bath with ammonium oxalate. The filtrates from the extraction were poured into absolute alcohol which precipitated pectin only from the plumule. The original residues of radicle and plumule were then boiled on a water-bath with 25 per cent. alcoholic hydrochloric acid, filtered, washed with alcohol, then re-extracted with 0.5 per cent. ammonium oxalate. On filtering, and pouring the extraction into alcohol, again a precipitate of pectic substance was given only by the plumules.

As it was not sufficiently clear that some of the pectic substance in the radicles was *only* removed after the protein of the wall had been loosened by Eau de Javelle, 1 grm. of radicles was extracted with successive quantities

\* Onslow, *loc. cit.*, p. 44.

of warm 0.5 per cent. ammonium oxalate until no further precipitate of pectin was obtained with alcohol. The residue was then left for 72 hours in Eau de Javelle, the solution filtered off and after washing well with distilled water, the radicles were again extracted with warm ammonium oxalate. The filtered solution poured into absolute alcohol gave a precipitate of pectin alone. The difference then, between the plumule and radicle is in the combination of pectic substances in the wall. Both have a certain amount of pectin directly soluble in warm ammonium oxalate. Both again have a protein-pectin complex from which the pectin is only removed after treatment with Eau de Javelle, but more is obtained from the radicles, and this apparently is all the pectin the radicles contain. But in the plumule there is yet more pectic substance, in close connection with some other constituent of the wall, probably the cellulose, which is partially removed after treatment with cold strong acid, but only completely removed after boiling acid.

The following micro-chemical experiments support the macro-chemical results. Fresh sections of the meristem of radicle, plumule and root do not stain with methylene blue. This would suggest that even the pectin, which is directly removed by warm ammonium oxalate, is combined in some way and cannot show the usual staining reactions. After 24 hours in Eau de Javelle the walls of section of both radicles and plumules stain intensely with methylene blue. After short boiling in ammonium oxalate the walls of the radicles still stain deeply. The walls of the plumule stain also except in the plerome. After treatment with Eau de Javelle followed by ammonium oxalate, sections of the radicle do not stain at all with methylene blue; the walls of the plumules are slightly stained except in the plerome. Sections of radicles or plumules warmed for 1 hour in aqueous caustic soda and giving a good cellulose reaction with chloriodide of zinc, do not stain with methylene blue, but sections boiled for 5 minutes in alcoholic caustic potash stain deeply, except in the plerome.

The prolonged treatment with aqueous alkali evidently removes the water soluble pectin, and probably also breaks up the protein-pectin complex, removing the pectin in the form of soluble salts, while in alcoholic solution the pectin remains. It seems possible that there is less pectic substance in the walls of the plerome, which are certainly thinner than the other walls of the radicle.

In the case of the plumule where the pectin is so firmly held, an alternative combination of the pectic substance might be as calcium pectate as in the middle lamella of the adult growing tissues, but this is negatived by the following microchemical observations. Longitudinal sections of the apical meristem of green and etiolated stems, of plumule and radicle, were left in

25 per cent. alcoholic hydrochloric acid for 24 hours, washed, then stained with methylene blue. On examination it was seen that:—

(1) In the green stem the middle lamella stained strongly in the walls behind the meristem but the walls of the meristem itself were entirely unstained.

(2) In the etiolated stem, while the middle lamella in adult tissues stained, the meristem remained unstained.

(3 and 4) Plumule and radicle—all walls quite unstained.

The pectin complexes of the meristems of radicle and plumule are apparently quite stable towards cold alcoholic hydrochloric acid, but on germination, behind the growing point the middle lamella is of calcium pectate, or possibly, in the case of the etiolated stem, another salt of pectic acid (see p. 126); these are decomposed by the acid, and the pectin is released, to stain subsequently with methylene blue.

In the region of the meristem then, there is in no case a middle lamella of calcium pectate, but the ease with which the cells macerate, even of the meristem, each separate cell being still surrounded by a definite organised wall showing considerable structure, suggests that a middle lamella is present here also, but different in composition from that of the older differentiated cells.

Maceration can be produced by:—

1. Treatment with Eau de Javelle followed or preceded by ammonia.
2. Cold concentrated sulphuric acid.
3. Boiling acids.
4. Boiling in lime water.
5. Boiling in 0.5 per cent. ammonium oxalate.

Eau de Javelle followed by ammonia, would remove protein as well as pectin—cold sulphuric acid would probably destroy protein—boiling acids, pectin—boiling lime water might precipitate both pectin and protein, but chiefly pectin, while ammonium oxalate gives further indication of pectin and protein, and negatives the assumption that the cementing substance is of fatty nature. Probably, therefore, the middle lamella in the meristem is a combination of pectin and protein, with relatively more protein present in the root and radicle meristem, and relatively more pectin in the plumule and shoot.

This is further supported by the results of an experiment on the digestion of the cell-contents by certain enzymes. When sections of radicle and plumule, after a two days' digestion with pepsin in acid solution, were transferred to a neutral solution of diastase for 48 hours, the sections were



easily macerated, due to the digestion of the protein by pepsin and of the pectin by pectinase, probably contained in the malt diastase (Haas and Hill (10), *loc. cit.*, p. 146).

#### IV. FATTY SUBSTANCES IN THE MERISTEM WALL, THEIR RELATION TO CALCIUM.

##### *A. Fatty Acids in the Meristem Wall.*

About 5 per cent. of the dry weight of the radicles of broad-beans consists of fatty substances extractable by boiling ether. These substances include the glycerides of saturated and unsaturated fatty acids and phytosterol. In addition, upon subsequent treatment with boiling alcohol, lipins giving characteristic myelin forms are extracted. These lipins are still under investigation.

In spite of this comparatively large percentage of fatty substances present in the radicles, the usual fat stains, Sudan III and osmic acid, give no clear indication of the distribution of these substances in a fresh section. Only after the disintegration of the protoplasts by Eau de Javelle and subsequent staining with Sudan III, do any definite red-stained bodies show in the meristem cells.

Czapek (8) has suggested a method for the microchemical investigation of the distribution of fat in the protoplasts; pyridine is used as a solvent to coalesce the finely dispersed "lipoid" into visible globules, which are stained by Sudan III dissolved in the solvent, the whole being mixed with tertiary amyl alcohol, to enable the penetration of the substance of the protoplast. With this method the cells of the meristem of the broad bean give obvious indication of these lipoids dispersed throughout the protoplasm.

On microtome sections various methods for staining and identifying fats have been tried (see Cranner, in Bolles Lee, *loc. cit.*, pp. 356-369 (3)). Fixing agents containing osmic acid such as Flemming have been found useful as indicating the probable distribution of unsaturated fats. Plumules, radicles and roots have been examined by this method, and although in the case of radicles and roots, blackened granules can be seen in the cells of the dermatogen in the developing leaves in the plumule, and in the vacuolated cells behind the growing point, in no case are these granules present in the cells of the meristem, which only show a diffuse fat stain with reagents containing osmic acid.

It is clear then, that the protoplasts of the meristem contain throughout their mass a considerable amount of "lipoid," including fats, phytosterol, and lipins, but in a fine state of dispersion. It is probable that these lipid substances may tend to accumulate at the boundary surface, if free to move,

and therefore form part of the substance of the wall from a very early stage. The recent papers of Hansteen Cranner (5, 6, and 7) have shown that fatty substances regularly form an integral part of the walls of normal parenchymatous cells. These considerations suggested that the failure of the meristem walls to give the cellulose reaction with chloriodide of zinc might be due to a combination of fatty substances with the cellulose. This would explain the fact that prolonged treatment of these meristems with aqueous or alcoholic alkali (p. 114) was followed by prompt reaction with chloriodide of zinc. The greater solubility of most potassium soaps in alcohol would account for the greater efficacy of alcoholic potash in preparing the way for the cellulose reagent.

The following experiment supplies further confirmation of the presence of fatty acids in the meristem wall. Twenty grams of radicles were finely ground and extracted with cold Eau de Javelle for seventy-two hours, the residue then filtered, washed well with distilled water and extracted for two hours with boiling absolute alcohol under a reflux condenser. The alcohol extract was filtered hot and immediately after filtering became very opalescent. The filtrate was concentrated and the precipitate that settled on cooling was filtered off and well washed with cold alcohol. This precipitate probably consisted mainly of lipins, but was completely used up in an unavailing examination for galactose, after hydrolysis with 1 per cent. sulphuric acid, as the presence of cerebrosides (Maclean (14)) was suspected.

The residue from the radicles was dried and then extracted with boiling ether (redistilled from potash) for two hours. The ether extract on evaporation only gave a very slight oily residue. After this treatment, it seemed certain that any uncombined fat had now been removed. The protoplasts had been dissolved out by the preliminary treatment with Eau de Javelle, and any fat subsequently extracted must come from the walls. The residue was now boiled under a reflux condenser for two hours with a 5 per cent. solution of caustic potash in absolute alcohol, the solution becoming deep yellow during the extraction. The extract was filtered hot, and when acidified with hydrochloric acid after the addition of water, a dense precipitate appeared which was filtered off and redissolved in absolute alcohol. On evaporation, 0.15 grm. of a brown fatty substance was obtained with an iodine value determined by Wijs' method (Leathes (12)) of 104. Glycerol could not be detected in the original alcoholic filtrate. When this fatty substance, which consists in part of an unsaturated fatty acid, is removed from the wall by saponification, the wall gives for the first time the cellulose reaction with chloriodide of zinc, from which it is assumed that the presence of the fatty acid in combination with the cellulose protects the cellulose from the action

of chloriodide of zinc. Protein and pectin were extracted from the residue by boiling aqueous hydrochloric acid, as described on p. 121.

It is probable that the plumule and etiolated stem possess a similar combination of cellulose and fatty acid, which is only dissociated by saponification. The removal appears to be a little more ready, and this is provisionally assigned to the fact that in these walls there is less of the protein complex attached to the wall and the fatty acid is more accessible to saponification. On the other hand, in the green stem, the wall gives the chloriodide of zinc reaction so much more readily that it is impossible to assume the fatty acid to be definitely combined with the cellulose. It is probably present in the wall in various combinations, in part as a calcium soap; hence the partial removal by cold concentrated hydrochloric acid (p. 120). This matter will be dealt with in the next section.

These conclusions as to the general distributions of fatty substances in the plant confirm the earlier findings of Hansteen Cranner, who gives evidence also for the presence of lipins of the lecithin type in the wall, but in the experimental work so far carried out, although lipins have frequently been found, no evidence has yet been obtained that shows these lipins to have been indubitably extracted from the wall and not from the substance of the protoplast. This seems to be true also of Cranner's experiments so far as at present described.

#### *B. Calcium in the Meristem and in Differentiated Tissue.*

The finely dispersed lipid material present throughout the meristematic protoplast, may be present as an inevitable bye-product in the active synthetic metabolism proceeding during growth (Priestley (18)), but there can be no question that its presence is of the utmost importance with reference to the entrance of water and solutes.

Some attention has, therefore, been paid to the distribution of calcium in the developing meristem, as undoubtedly the arrival of inorganic kations and notably of calcium, will have a most important bearing upon the lipid-water relation of the protoplast. The experimental results obtained are given in this section; unfortunately the macrochemical data suffer from our inability to distinguish between calcium present in the wall or in the protoplast, but the data given have a certain significance in comparison with the amounts of calcium present in differentiated tissue, although it must be remembered that quite arbitrary limits between these tissues have to be chosen in isolating material for analysis. As a consequence the maceration experiments given at the end of the third section (p. 121) have perhaps greater significance.

The lipoids are dispersed throughout the protoplast, probably as a very

finely divided and permanent emulsion, either an emulsion of oil in water or of water in oil. It has already been suggested (Priestley and Tupper-Carey (20)), that in the very early stage the meristem cell is practically impermeable to water and this would suggest a water-in-oil emulsion. Such an emulsion is quite possible and its stability would depend upon an emulsifying agent, that is a substance lowering surface-tension at the water-and-oil interface, which is more soluble in the oil than in the water (Bancroft (2), *loc. cit.*, p. 262).

Such an emulsifying agent is a calcium soap, while excess of potassium and sodium soaps, more soluble in water than in oil, would favour the formation of an oil-in-water emulsion, which would presently leave the protoplast readily permeable to water. Continued synthetic activity would then be carried on under greater disabilities.

Palladin's (17) observations are most suggestive in this connection. He placed the yellow leaves from 18-day old etiolated plants of *Vicia Faba*, L., in the light, in various nutrient solutions. He found that in distilled water, 0.3 per cent. calcium nitrate solution, and also in a 10 per cent. solution of cane sugar, the leaves all died, but that in 10 per cent. cane sugar, plus 0.3 per cent. calcium nitrate, they remained quite healthy. Other observations (Priestley and Ewing (21)) have given reasons for regarding the rudimentary leaf on an etiolated broad-bean as practically nothing but meristem, it is therefore striking that when the leaf is provided with organic nutriment, plus a calcium salt, it is able to live and remain healthy.

The meristematic protoplast in presence of the nutrient sap diffusing along the walls, does not remain in continuous equilibrium with it, and one reason for the change involved in differentiation may well be the constant loss of substance to the wall from the protoplast. This loss is probably the result of precipitation; soluble salts will attain an equilibrium, but it is quite otherwise if these salts, whether pectate or soap, are left on the wall in an insoluble form. Then in the meristem cells nearest the supply of sap, the deposit of calcium salts as it proceeds, will hasten the time when the ectoplast, drained of lipid material, loses its impermeability and, with the formation of bye-products having high osmotic power, allows the passage of water into the distending vacuoles within. Thus, if a trace of calcium enables the meristem cell to maintain its internal conditions so that they are suitable for synthetic metabolism, a continuous supply results in increased permeability and subsequent differentiation.

This assumption is strengthened by the following experiments:—The amount of calcium present in the various meristems was estimated by its

precipitation as calcium oxalate and the subsequent titration of the free oxalic acid with potassium permanganate by a method described by A. T. Shohl and F. C. Pedley (24), with the exception that it was found necessary first to ash the dried material in a platinum crucible and then extract the residue with dilute hydrochloric acid. The percentage of calcium is given on the dry weight—green stem apices of broad-beans grown in the light contained 0.015 gm. per cent., while the apices of etiolated stem contain 0.0103 gm. per cent. A reverse difference was noticed when the dried material was extracted for 24 hours with distilled water. Here the extract from the green stem apices contained an average percentage of 0.004 gm. of calcium and the apices of the etiolated stems 0.005 gm. per cent., which suggests that the etiolated stem apex contains more calcium uncombined with pectin or fatty acid, and therefore directly extractable with water. The dried radicles from the broad-bean seed contain 0.0086 gm. per cent. of calcium. The tips of growing primary roots contain 0.012 gm. per cent., while primary roots without tips contain 0.014 gm. per cent. That roots and root tips show a higher percentage than stem apices is probably due to their position with regard to the source of calcium, and to their absorptive activities.

#### *Discussion.*

In this section an attempt is made to sum up the results without further discussion of their experimental basis, so as to indicate their bearing upon the development of the plant wall and upon the chemical and developmental differences between the various apical meristems. In view of the complexity of the problem, only the broadest and the most general statement is attempted. In the first place, it is assumed that the wall arises by gradual transition from a living protoplasmic interface to a series of relatively simple chemical compounds such as pectin, cellulose, fats, etc.

Historically, the development of the plant wall has been the subject of much controversy, but from a morphological point of view, and the opposing schools both seem to supply evidence in support of the preceding statement. Thus Wiesner (28) and Strasburger (25) support its protoplasmic origin, while Gleisberg (9) has recently reviewed its evolution from a protoplasmic-protein surface to carbohydrate lamellæ, through various phylogenetic series of organisms. This review reveals the striking general similarity as to wall structure that exists between a simple colony of cells embedded in a common pectin gel, and the highly organised cells of the vascular plant, where the loose gel is replaced by the firm cement of the salts of pectic and fatty acids.

Incidentally, the long controversy aroused by Wiesner's views as to the

existence of protoplasm in the cell wall, has led to the accumulation of a considerable amount of microchemical evidence for the presence of proteins or their decomposition products, in the cell wall. The observations of Zacharias on the growth of root hairs, quoted by Strasburger, have demonstrated the presence of a substance staining only brown with chloriodide of zinc, that precedes the formation of cellulose, and Krabbe's (11) work upon the development of sclerenchyma, points in the same direction. Even such a whole-hearted opponent of Wiesner as Correns (4) has supplied evidence for the presence of tyrosin in the wall. Such microchemical evidence, though insufficient alone, may carry conviction when supported by the macrochemical results in the preceding sections.

If the meristem wall always commences as an essentially protein interface between two masses of protoplasm, the outstanding problem is to explain the difference in the walls of the meristems of stem and root, assuming this similar starting point for both, whilst the final product of differentiation is usually very similar also. When a normal parenchymatous tissue has formed behind the growing apex, whether of root or stem, the walls of the parenchyma give similar microchemical reactions; in both cases cellulose is a main constituent of the inner wall and calcium pectate of the middle lamella, but this final result has evidently been attained in different ways, which may be associated with the different metabolism of the protoplasts in the two meristems. Lack of information prevents any attempt at explanation of the difference in terms of metabolism, and for the moment, the simplest method of stating the facts in a generalised manner, appears to be found in the assumption that in the growing point of the root the newly-formed walls of the meristem are more conservative in character, and retain their protein constituents and more complex chemical character for a longer time than the walls of the stem meristem. In the latter the transition to an essentially more heterogeneous, but less chemically complex system, takes place within the region of the meristem itself. As a result, the walls of the meristem region in root and stem show important chemical differences which may be briefly resumed as follows:—

The meristem walls of radicle and root apex are not far removed from the protoplasmic stage, and consist mainly of a protein-pectin and protein-cellulose complex, the cellulose also linked with fatty acid and with some pectin present less firmly combined and possibly not in the wall. The meristem walls of the plumule still contain the protein-pectin complex, but associated with a cellulose-pectin fatty acid complex. After a broad-bean has been soaked for 24 hours in water, the meristem walls of the plumule give the cellulose reaction with iodine and sulphuric acid, which is most simply

explained as due to the loss of protein. The meristem walls of the etiolated stem seem therefore to be characterised mainly by a cellulose-pectin fatty acid complex. In the normal green stem apex this complex seems to be merely a cellulose-pectin, with possibly a certain amount of fatty acid as the soap of an inorganic kation. The middle lamella in the meristem is never found to be of calcium pectate, but probably as the separation of these complex molecules takes place in the adjacent wall the protein and pectin appear as the middle lamella.

Expressing the whole of these changes schematically we have:

MERISTEM.				ADULT PARENCHYMA.		
	Radicle and root.	Plumule.	Etiolated shoot.	Green shoot.	Etiolated shoot.	Root and green shoot.
WALL	PROTEIN-Pectin	PECTIN-Protein	PECTIN	PECTIN	Pectin	Pectin.
	CELLULOSE	CELLULOSE	CELLULOSE	CELLULOSE	CELLULOSE	CELLULOSE
	Fatty acid	Fatty acid	Fatty acid	Fatty acid and soap	Fatty acid and soap	Fatty acid or soap.
MIDDLE LAMELLA	PROTEIN	Protein	Protein	Protein	PECTIN	Calcium pectate
	Pectin	PECTIN	PECTIN	PECTIN	Fatty acid	and Calcium soap.

No attempt has yet been made to trace these series of changes in the wall to a causal developmental mechanism, which will involve a study of the enzyme catalysts present, especially protease, pectase and lipase. Some preliminary observations of the action of pepsin upon the meristem walls of the root, show that they are very appreciably digested by this enzyme in a medium of suitable reaction. As the reaction of the sap at root and stem apex is usually different, a different rate of progress in the developmental stages of the wall at the two apices would probably result.

In conclusion it may be pointed out that the data accumulated in this paper are not in disagreement with the conclusions reached on other grounds previously (20), as to the diffusion of sap between the protoplasts of these different meristems. The more chemically complicated and less differentiated the wall, the more impermeable, presumably, it will be, considering its derivation from the impermeable meristematic protoplast. As differentiation proceeds and cell-wall and middle lamella consist more exclusively of carbohydrates and fatty acids, aqueous diffusion takes place more freely, pectic and fatty acids are precipitated as calcium salts and the wall's power of absorbing and retaining water is also diminished (Cranner (7)).

*Summary.*

1. Differences in microchemical reaction between the apical meristems of root and stem in the broad bean, suggested that a difference in chemical composition might exist and explain the difference in relative permeability found during an earlier investigation.

2. A detailed microchemical investigation of the reaction of the cell walls of the various meristems towards cellulose reagents led to their classification as follows:—

(1) *Radicle, root and plumule*, which do not give the cellulose reaction with iodine and sulphuric acid unless previously treated with strong acids or alkalis, and do not give the cellulose reaction with chloriodide of zinc unless well treated with aqueous or alcoholic alkalis.

(2) *Etiolated stem*, which gives the reaction with iodine and sulphuric acid direct, but require short treatment with boiling aqueous or alcoholic caustic alkalis before the reaction is given with chloriodide of zinc.

(3) *Normal green stem*, which gives the reaction with iodine and sulphuric acid direct, and only a short treatment with various reagents is necessary before the reaction is given with chloriodide of zinc.

3. Macrochemical experiments prove the existence of cellulose in the walls of the meristem, but its presence is masked by association with other substances.

4. Protein, closely linked to the cellulose, is found by macrochemical experiments to be most probably the substance which prevents the reaction with iodine and sulphuric acid.

5. Pectin is present in each case, though not directly linked to the cellulose in the meristem wall of radicle and root.

6. The middle lamella in the meristem is never of calcium pectate but is probably a mixture of pectin and protein.

7. When all fat has been extracted from the meristem of the radicle by the use of the usual fat solvents, and the protoplasts themselves dissolved out with Eau de Javelle, the residue, after boiling with alcoholic potash, yields a small quantity of some fatty substance, presumably from the walls, with an iodine value of 104.

This fat, present in the walls of the meristem of root, radicle, plumule and etiolated stem, closely linked to the cellulose, is responsible for the failure of chloriodide of zinc to give the cellulose reaction.

8. As the impermeability of the meristematic protoplast depends, in part, upon the fats present in a finely divided state, the presence of calcium in the



## 130 *Composition of Cell-Wall at Apical Meristem of Stem and Root.*

sap will cause precipitation, involving increased permeability and subsequent differentiation.

9. Estimation of the amount of calcium present in the various meristems show it to be greatest in the stem apex when grown in the light and least in the meristem of the radicle.

10. It is assumed that the plant wall arises by gradual transition from a living protoplasmic interface, to a structural system of relatively simple chemical compounds.

Various writers incidentally support this view, so that the difference in chemical composition of the cell walls at the various meristems is explained by the suggestion that the developmental changes proceed most slowly at the root apex, more quickly in the plumule and etiolated stem, and most rapidly at the normal green stem apex, under the influence of enzyme catalysts.

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*On the Effect of X-rays of Different Wave-Lengths upon some Animal Tissues.—Proof of Differential Action.*

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(Communicated by Prof. A. W. Porter, F.R.S. Received April 17th, 1923.)

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*Introduction.*

In a paper (1) upon the Germicidal Action of Ultra-Violet rays, it is shewn that a marked differential action exists in this part of the spectrum, i.e., electromagnetic disturbances have a different effect upon the same kind of organism

according to the wave-length operating. It was found that when micro-organisms were exposed to the ultra-violet and visible radiation from a tungsten arc for a considerable period of time, germicidal action stopped abruptly at  $\lambda = 2960 \text{ \AA.U.}$  It was possible in this particular instance to correlate the differential action with a selective absorption by the micro-organisms of just those wave-lengths which had germicidal action upon them.

In attempting to extend this kind of experiment into the region of X-rays, it soon becomes certain from ordinary electroscopic absorption measurements that this correlation, even if it exists, will not be found, because the absorptive power exhibited by a medium upon a beam of X-rays is dependent in so many cases solely upon the mean density of that medium. Hence if we take a section of some animal tissue the density of which is about the same as water, the absorption which it will exert upon a beam of X-rays will be practically the same as that of a layer of water of equal thickness, in spite of the fact that in the layer of tissue the composition varies from point to point according to the cellular structure involved. In order to reveal this point-to-point absorption it would be necessary to construct electroscopic instruments on a microscopic scale.

In view of these difficulties, it was decided to select X-rays of a certain restricted range of wave-length, and, in the first place, to expose tissues to measured doses of this radiation. The next step was to go considerably higher in the scale of wave-length, arrange that exactly the same dose of radiation was given and see whether the same effects were produced. If different, then it would be proved that a differential effect exists; and these experiments support this view. The proviso is inserted here that this differential effect may really be due to a selective absorption of the radiation by some constituent part of the cells, which cannot of course be detected by the usual absorption measurements.

These experiments have been carried out on the normal skin of the rat and Jensen's Rat Sarcoma which, when inoculated subcutaneously, grows progressively in a large percentage of cases.

It has been shewn in previous papers that the rate of growth of these tumours can be slowed up or completely stopped by accurately adjusting the dose of radiation; if growth is permanently stopped we say that a lethal dose has been given.

## 2. *Physical Section.*

In view of the fact that proof of a differential action will depend almost entirely upon the reliability of the physical methods used for measuring the

radiation, the subject matter of this paper is divided into two sections, the first of which is devoted to certain physical considerations.

*The Ranges of Radiation used.*—The ranges of X-ray wave-lengths used have been chosen so that it may be possible to draw conclusions from the observations which will be applicable to radio-therapy. One group of wave-lengths used was that which was emitted by an X-ray tube running at an alternative spark gap of 4 cms. between spheres 5 cms. in diameter; the wave-lengths would range for the most part from about 0.45 to 0.30 Å.U. and reference will be made to them in the text as “soft” X-rays. The other group chosen was a comparatively small group of wave-lengths obtained by running the X-ray tube at an alternative spark-gap of 10 cms. between the spheres and screening the radiation by 1 cm. of aluminium. Under these conditions the transmitted rays are not far from being homogeneous, with a wave-length of 0.168 Å.U.; reference will be made to them as “hard” X-rays. The figures relating to wave-lengths are derived from aluminium absorption measurements.

*Definition of the Dose of Radiation.*—In this paper it has been decided to take the *dose* to be measured by the amount of radiation *absorbed* by the tissue and it will be referred to in the Animal Experiments' Section as the *absorption dose*. This is different from the usual practice, which is to take the dose as measured by the product of Incident Intensity and Time, where the incident intensity  $I$  is the amount of energy incident per second per unit area.

The absorbed radiation  $A = I(1 - e^{-\mu x})$  where  $\mu$  is the coefficient of absorption and  $x$  the thickness of tissue. When the wave-length is changed the value of  $\mu$  is in general different, so that  $A$  and  $I$  are not in a constant ratio to one another for different kinds of radiation.

In a similar way an electroscope does not measure the energy incident upon it, but only that which is absorbed in it and used to produce ionisation. We can represent this fraction approximately by  $A_0 = I(1 - e^{-\mu_0 x_0})$ . It is the quantity  $A_0$  which is measured by the *reading* of the instrument. If we assume that  $A_0$  bears a simple proportion to the energy absorbed by the tissue ( $A$ ), and that this ratio is the same for both “hard” and “soft” rays, then the readings on the electroscope multiplied into the time measure the *dose*.

*Experimental Method of Measuring the Dose.*—In applying the method outlined we have to design the electroscope so that the above assumptions shall be most nearly satisfied. It is well known that when a beam of X-rays meets an obstacle, secondary radiation originates and the general character of this radiation changes as the incident wave-length is varied. The resulting ionisation in, say an air space, depends upon the general character of the radiation

traversing it, so it is clear that the simpler we are able to keep the conditions the more likely we are to avoid the various complications of secondary radiation. For this reason a short air path is used as our standard ionisation chamber and it is arranged so that in the passage of the beams of X-rays through this air path no solid surfaces are struck. In this way secondary and scattered radiation effects are reduced to a minimum, and we can then assume that when an equal ionisation is produced by two beams of different wave-lengths, there is an equal expenditure of energy producing such ionisation in the two cases.

The experimental method of measuring the dose of radiation may be seen from fig. 1. X-rays originating at A go along the paths indicated, the vertical



FIG. 1.

beam to an ionisation chamber N connected to an electroscope, which will be called the Near electroscope; the horizontal beam to an ordinary gold-leaf electroscope situated in a room about 4 metres away; this will be called the Far electroscope. N could be swung in and out of position, and this position, when it was receiving the vertical beam, was that occupied by the experimental material; in this way a measure was always obtained of the radiation to be used, simultaneous measurements being taken at F.

When the actual exposures of the tissues took place, N had to be swung out of position, but as there had previously been simultaneous readings on F and N, it was not a difficult matter to ensure that the radiation was of the character and intensity required, for during the exposures, which in some cases lasted over an hour, observations of the intensity at F were made every minute; in this way an invaluable check upon the constancy of the radiation was obtained, and any variations in it were made good by a slight lengthening or shortening of the exposure. F and N were standardised before use on all occasions by means of a known quantity of radium.

*Effect of Variations in the Ionisation Chamber.*—It has already been mentioned that the form of ionisation chamber at N which we look upon as the standard consisted of a simple air path. This was obtained by taking a lead cylinder

and cutting a small hole in it transversely to its length. Two electrodes were set up vertically within the cylinder, so that the beam of X-rays came down between them but without touching them; one was connected by means of a wire to a gold leaf electroscope about 6 feet away; the other terminal was earthed. The saturation current obtained when X-rays of a certain wave-length traversed the air gap was taken to be proportional to the quantity of radiation absorbed. The actual reading when multiplied by the time of exposure constitutes a measure of the dose. When used for measuring X-rays of a different wave-length the output can be adjusted to give the same reading on the electroscope, and so equal doses can be given.

The ionisation curves obtained with this air chamber for the two regions of wave-length under investigation are practically identical; see fig. 2, in which the crosses correspond to "hard" rays and the circles to "soft" rays. This

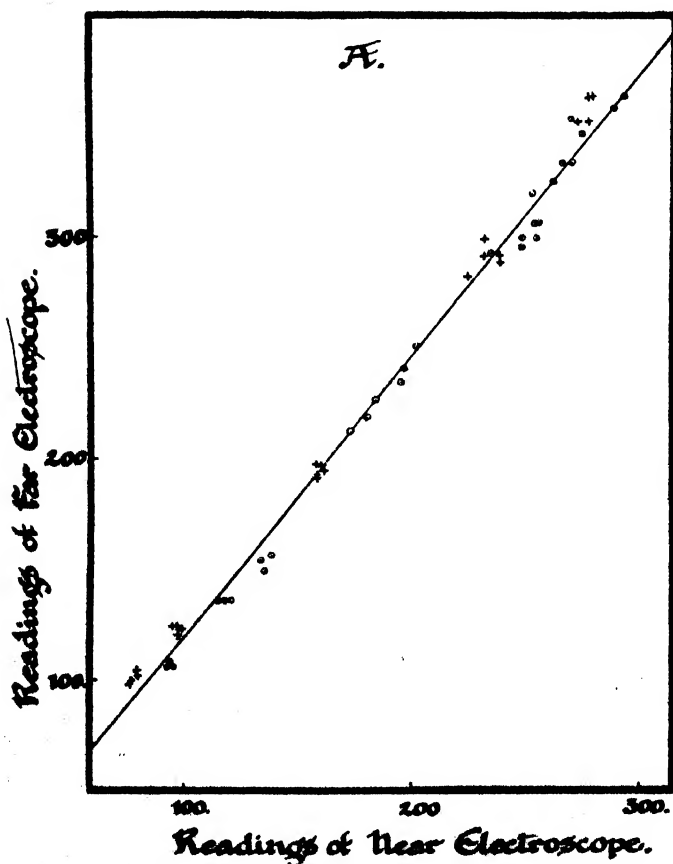


FIG. 2.

is a graph of a number of simultaneous observations on the Near and Far Electroscopes; by using a diaphragm in front of F it was possible to get identical readings on F with "hard" and "soft," when these beams were giving identical readings on N.

Various ionisation chambers were used and the effect of varying the ionisation chamber at N is shewn in fig. 3. These three graphs were obtained in the

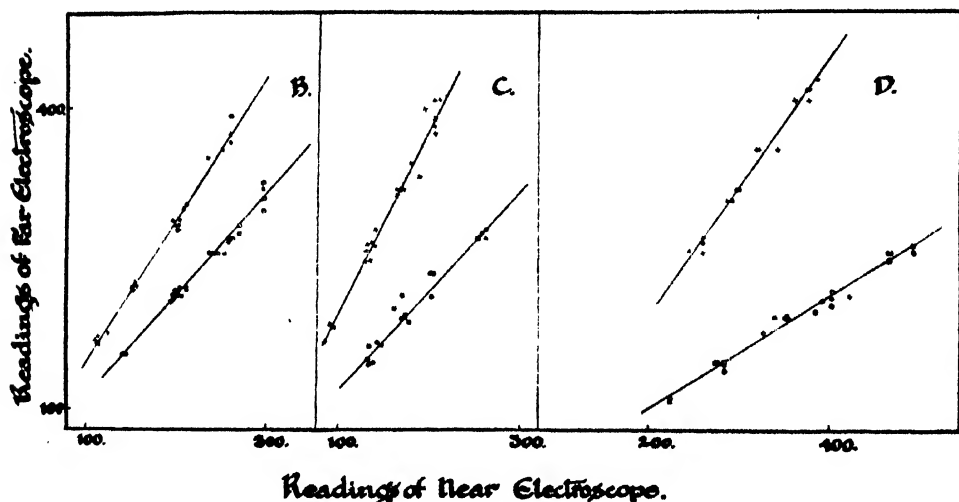


FIG. 3.

manner just described; the ionisation chamber in (b) was a shallow wooden box with a central electrode of wood, in (c) it was the same box, but the central electrode was of aluminium, and in (d) it was a shallow aluminium box with an electrode of the same metal. The graphs shew that when equal readings are obtained on the Near electroscope, the readings of the Far one are higher when dealing with "hard" than with "soft" X-rays, except when the air ionisation chamber is used.

Table 1 shews the extent to which this is the case. It follows that when we use, say, chamber (d), and adjust the readings of N to equality for "hard" and "soft" rays, we are really supplying in equal times doses for "hard" rays 2.3 times as great as for "soft" rays. It might be possible by using a measuring instrument which, owing to secondary radiation phenomena, specially favours "soft" X-rays, that the existence of a differential effect upon animal tissues could be entirely masked. It is not unlikely that these phenomena have played some part in the observations of Friedrichs (2), from which he has concluded that no differential effect upon tissues exists.

Table 1.

Ionisation chamber used at N.	Ratios of readings of Far and Near Electroscopes for different rays when Near Electroscopes gives uniform readings.
(a) Air Ionisation chamber only..	
(b) Wood Ionisation chamber : wood electrode	1.37
(c) Wood Ionisation chamber : aluminium electrode .....	1.73
(d) Aluminium Ionisation chamber : aluminium electrode .....	2.03
(e) Paper Ionisation chamber : aluminium electrode .....	2.52

In view of the fact that the constructional features of the ionisation chamber may account for such important differences as appear in Table 1, details are given below of the dimensions of (a), (b), (c), &c. They are as follows :—

- (a) Air gap only—dimensions of gap about  $1 \times 0.4 \times 2$  cms.
- (b) Rectangular parallelepiped of wood, external dimensions 3.4 cms. long, 1.5 cms. wide and 0.4 cms. deep, thickness of wood 0.3 mm.
- (c) Same as (b), but electrode of aluminium 0.2 mm. thick.
- (d) Same dimensions as (b), but all of aluminium 0.3 mm. thick.
- (e) Cylinder of thin paper about 0.8 mm. thick length 3.5 cms., diameter 1.5 cms., central electrode an aluminium wire.

### 3. *Animal Experiments Section.*

*Experiments upon Tumours.*—For the tumour experiments the method of exposure was that adopted by Wedd and Russ (3). A thin slice of tumour about 1-2 mm. thick is placed between thin sterile mica sheets and exposed to the radiation for the length of time desired ; small pieces of the tumour are then inoculated into normal rats and the subsequent growth determined by frequent caliper measurements. Here again the animal variation has been eliminated as far as possible in the following way. The experimental batches of animals inoculated numbered about twenty, and each rat was usually inoculated with three small pieces of tumour ; one had been exposed to “hard” rays, one to “soft,” and the control had had no radiation at all.

The first step was to find the dose of “soft” X-rays which was just sufficient to produce a lethal effect upon the tumour ; this gave a certain rate upon the



Standard electroscope at N (fig. 1), and this rate could be accurately duplicated with "hard" X-rays and the corresponding dose of these rays could be given to the tumour.

Two pieces cut from the same tumour were exposed, one to an absorption dose, which was practically a lethal dose, of "soft" X-rays (exposure 53 minutes), and the other to an equal absorption dose of "hard" X-rays (exposure also 53 minutes). Small portions of the irradiated tumour were then inoculated into the right and left axillæ of 20 rats and non-irradiated tumour in the right groin. The chart A, fig. 4, shews the result three weeks later, from which it will be seen that although the absorption dose of "soft" X-rays was lethal to

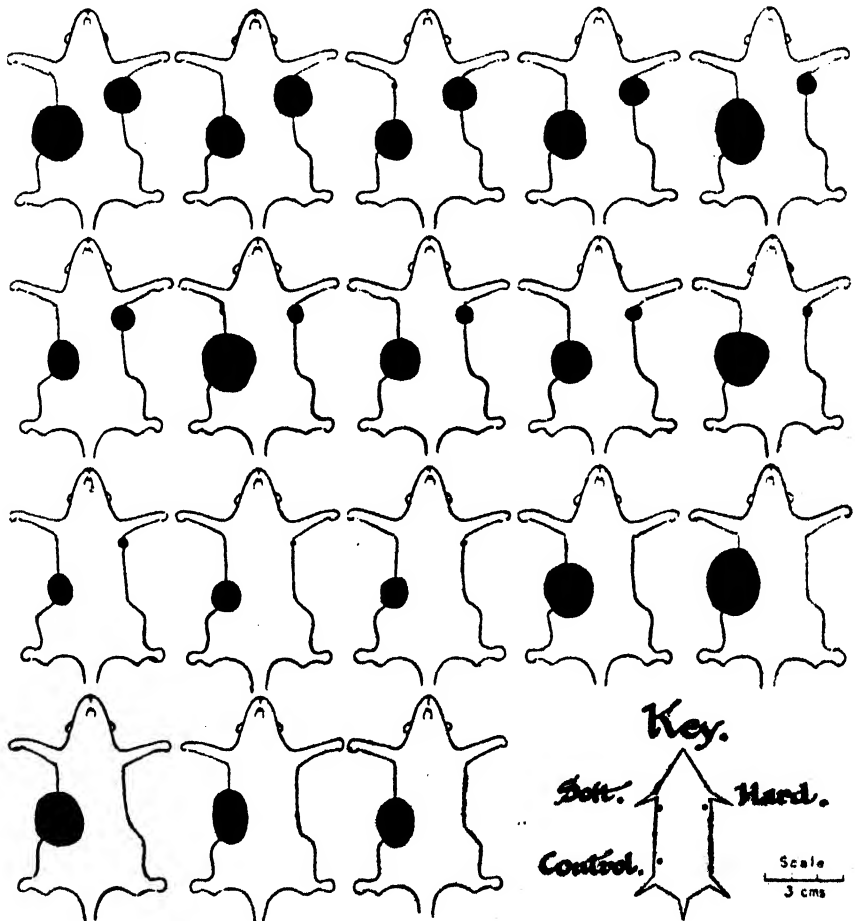


FIG. 4.

the tumour, an equal absorption dose of "hard" X-rays resulted in a very considerable degree of growth. The tumours are drawn to full-scale.

In the next series of experiments upon the tumour cells it was arranged to find out how much greater an absorbed dose of "hard" X-rays it was necessary to give in order to get the same effect as with "soft" rays; the effect sought was a lethal effect upon the tumour.

The results in Table 2 were obtained under these conditions. For any particular time of exposure the absorbed dose of "hard" rays was 2.53 times as great as that of the absorbed dose of "soft" rays; in spite of this increase in the amount of X-ray energy absorbed it will be seen that the lethal effect was reached a little sooner with the softer radiation, so that this number would not appear to be quite large enough as a measure of the differential action.

Another experimental series undertaken, so that the absorbed dose of "hard" rays was 2.74 times as great as that of the "soft" rays, shewed that in this case the lethal effect was obtained a little *sooner* with the "hard" than with the "soft" rays.

Two further series on the same lines shewed that the lethal effect upon the tumour was obtained with "hard" and "soft" rays when the absorbed dose of "hard" rays was in one case 2.85 and in the other 2.42 times as large as the absorbed dose of "soft" rays.

Table 2.

Number of animals.	Number of control inoculations which grew tumours.	Time of exposure of the tumour.	Tumour inoculations which grew after exposure to—	
			"Soft" X-rays.	"Hard" X-rays.
12	9	39 minutes	3	9
22	18	50 minutes	4	11
12	11	56 minutes	1	4
12	8	61 minutes	0	1

If we take the whole series into consideration, involving experiments and observations upon about 300 rats, it appears that when a quantity of energy is expended in a layer of tumour by a beam of what is here termed "soft" X-rays, then in order to get the same degree of action upon the tumour (namely prevention of growth) with a beam of what is here termed "hard" X-rays, it is necessary to arrange that about 2.6 times (average of the numbers in the text)

as much energy is expended. This number may therefore be looked upon as a numerical measure of the degree of the Differential Action, and might be termed the Therapeutic Factor.

*Skin Experiments.*—In the experiments upon the normal skin the rats were given urethane and exposed to a beam of X-rays projected vertically downwards. A lead screen with a square aperture was placed over the animal to localise the exposure. The screen was first placed in such a way that the aperture came between the shoulders of the animal and a dose of “soft” X-rays given to this area; the screen was then moved so that the aperture came low down in the mid line and a measured dose of “hard” X-rays given to this area. By exposing two areas of the skin of the same rat, comparisons between the effects of “hard” and “soft” X-rays were made more accurate. The rats were generally irradiated in pairs and in order to exclude “local” variations the upper area of one rat and lower area of the other were exposed to the same wave-lengths.

When an animal is irradiated in this way, the radiation which is scattered back from the body of the animal as the primary beam makes its way through it, contributes something to the dose absorbed by the skin. In order to allow for this fraction, when the dose of radiation was measured, a rat was placed just beneath the electroscope so that the back radiation could contribute to the ionisation produced by the direct beam. A difference could be measured with the animal in or out of position, but it only amounted to a few per cent. of the total ionisation.

After the X-ray exposure, a careful scrutiny was kept of the successive changes in the skin. In some cases the exposures were long enough to produce ulcers which eventually healed; with shorter exposures, the irradiated region was not damaged enough to prevent the re-growth of hair, though in most of these cases there was a loss of pigment.

A preliminary series of experiments upon 18 rats on the lines indicated served to shew that in order to get the same degree of reaction in the skin it was necessary to give a considerably greater absorbed dose of “hard” than “soft” X-rays.

The extent of this differential action was more accurately gauged from some further results obtained after the exposure of another series of 48 rats to measured doses of the two types of radiation.

Detailed records were kept of the times at which the various reactions occurred, and in many cases the observations were continued over a period of nearly a year.

The times of exposure to the “soft” rays ranged from 21–50 minutes and to

the "hard" rays from 30-150 minutes, and it was found that a practically identical degree of reaction was obtained in the skin of the rats when the absorbed dose of "hard" X-rays was six times that of the "soft"; this number may therefore be taken as a measure of the differential action of the two sets of wave-lengths. If we use the term Therapeutic Factor (*vide* p. 140) it is seen that a considerable difference is found for skin and for tumour.

In several cases where the general degree of reaction of the skin to "hard" and "soft" rays was about the same (produced, however, by widely different doses) there still seemed some small difference in the nature of the reaction. With the "hard" X-rays the onset of the reaction was slower, and the condition of the hair many months later was nearer the normal.

#### 4. *Discussion of Results.*

If the method by which comparison is made of the amounts of energy liberated in the tissues by the two beams of X-rays under consideration is a valid one, then the results shew that when equal amounts of X-ray energy are absorbed from beams of different wave-lengths by animal tissues, the reactions of these tissues may differ considerably in degree and possibly to some extent in nature.

The conclusions reached have some bearing in radio-therapy. The experiments shew that a bigger differential effect between "hard" and "soft" X-rays exists for the skin of the rat than for the tumour cells. Provided this also holds for the human subject it is one argument for the use of very hard X-rays or  $\gamma$ -rays when tumours at a considerable depth below the skin have to be irradiated. The conclusion would perhaps be just the reverse in superficial conditions not involving the irradiation of normal skin.

The result also raises the question whether the specification which has been adopted by Seitz and Wintz for expressing the carcinoma dose in terms of the skin dose is sufficiently rigorous, for this may be a function of the wave-length.

The two main results obtained are that about six times as much short wave-length energy as long wave-length energy must be expended in a layer of skin in order to produce equal reactions and that this factor falls to about 2.6 in the case of the tumour. We may question whether these are not cases in which the molecular configuration, apart from the atomic, is more affected by electrons of low than of high velocity. The experiments of C. T. R. Wilson have produced direct evidence of the part which the electrons liberated in the track of a beam of X-rays play in ionisation, and it would not be irrational to suppose that the slower electrons which would be released by the longer wave-length

can give rise to more pronounced effects in the tissues than the quicker ones, even though a similar expenditure of energy occurs in the two cases.

#### LITERATURE.

- (1) Browning and Russ. 'Proc. Roy. Soc.,' B. vol., p. 90 (1917).
- (2) Friedrich, 'Physikalische und biologische Grundlagen der Strahlentherapie' (1918.)
- (3) Wedd and Russ, 'Journ. Pathology and Bacteriology,' vol. 17, p. 1 (1912-13).
- (4) Seitz and Wintz, 'Die Röntgentiefentherapie' (1920).
- (5) Wilson, 'Proc. Roy. Soc.,' A. vol., p. 85 (1911).

### *Stimulus Rhythm in Reflex Tetanic Contraction.*

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(PLATES 7-10.)

When an afferent nerve is stimulated by single shocks following each other in not too fast succession the resulting reflex tetanus shows tensile vibrations corresponding with the rhythm of the stimulation. We find that the upper limit of stimulus frequency at which incompleteness of fusion of the component reflex contractions is still traceable differs considerably for different types of reflex.

*Method.*—Our observations have been upon the mammalian preparation (cat) in the decerebrate condition. Transection of the brain-stem between the anterior and posterior colliculi was performed under complete chloroform-anaesthesia, and the whole of the brain anterior to the transection removed. For purely spinal reflexes the level of the spinal cord transection lay variously between 12th thoracic and 3rd lumbar segments, the preparation being also decerebrate. In some instances the spinal section was performed, under full anaesthesia and asepsis, four to eight days prior to the decerebration. For use with the myograph the contracting muscle was in all cases isolatedly attached to it, and all other muscles in both hind limbs immobilized by appropriate nerve-section or resection. Fixation of the limb was obtained by steel drills in femur and tibia, a strong clamp holding the pelvis. For stimulation induction shocks were used, these being (except where otherwise stated) single shocks at frequencies varying from 35-95 per sec. For obtaining single-shock series of

these frequencies a special key (*see* Appendix) had been devised (C.S.S.) separating the make- and break-shocks of the ordinary inductorium, from which its spring interrupter had been removed. For most of the observations the primary coil was coreless, and the break-shock was given by short circuiting the primary coil, the current in the unshorted primary being usually 70 milliamps. The stimulating electrodes were 10 mm. apart with anode in all cases toward the cut end of the stimulated nerve. The isometric myograph was similar to that previously described. Its vibration rate when unattached to the muscle was over 1000 per sec.

*Observations.*—The muscles used for the reflex contractions have been the ankle-flexor, tibialis anticus, a knee-flexor, semitendinosus, and the knee-extensor, quadriceps femoris. The difference found, as mentioned above, between the fusion frequencies of the reflex tetani of the various type-reflexes examined is not explicable by difference between the motor-nerve-muscle reactions (mn. tetani) of these muscles (*fig. 1*).<sup>\*</sup> Under direct motor-nerve stimulation all these muscles when similarly examined by the myograph showed in their tetani at 50 single shocks per sec. about the same degree of incompleteness of fusion of the individual contraction waves, and both with tib. ant. and vasto-crureus the individual waves were sometimes traceable to 90 per sec. with break-shock series of that frequency (*fig. 2*, *see* ascent corner). There may exist some differences between these several muscles in regard to their summation frequencies, but such differences are small as compared with those we observe in their reflex tetani examined in various type-reflexes. Our observations suggest that vasto-crureus has a rather lower summation frequency than has tibialis anticus.

We have noticed not seldom in mn. tetani at frequencies somewhat too low to cause complete fusion of the individual contraction waves, *e.g.* with 50 per sec. for tibialis anticus, that the tremor although well seen in the records with maximal tetani is lost with stimuli of intensity above maximal, so that the myogram then presents a perfectly smooth line: and sometimes when these stimuli are not very far above the strength by which maximal tetani are just attained. We have seen this occur with mn. tetani of semitendinosus even when the frequency has been as low as 36 per sec. The explanation may lie in the "repetitive firing" of the nerve-fibre noted by Forbes and Gregg† to

\* The scale against which tensions are marked is in millimetres and centimetres on the original records; the magnifications apply to the originals.

† Forbes, A., and Gregg, A., 'American Journ. of Physiology,' Vol. XXXVII, p. 118 (1915); and *ibid.*, Vol. XXXIX, p. 172 (1916).

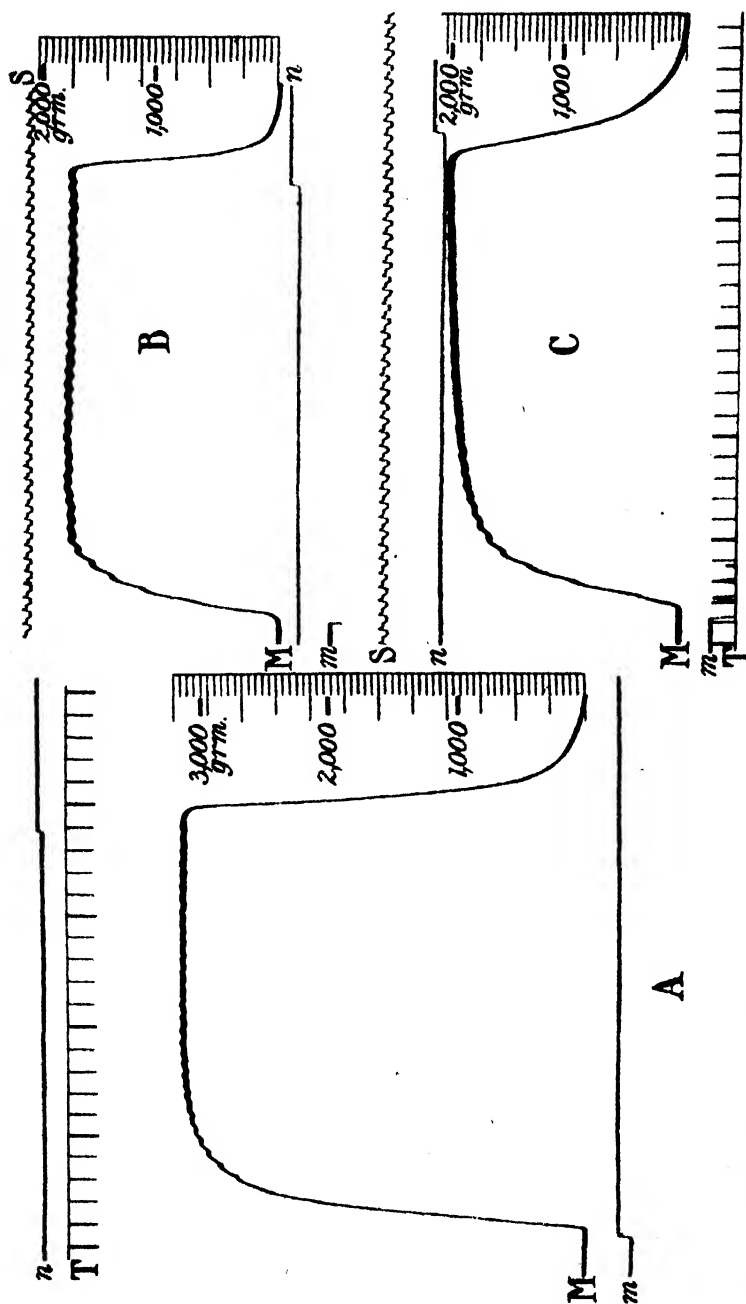


FIG. 1.—Tetanus from motor nerve. A. *Semitendinosus*: break shocks at 50 per sec.; B. *Tibialis anticus*: break-shocks at 38 per sec.; C. *Vasto-crureus*: break-shocks at 38 per sec. M, myograph; T, time in  $1/25$  sec.; m. and n. unshorting and shorting of stimulating circuit; S, stimulating key. Scale of contractile tension in grms. Tendon movement  $\times 35$  in A;  $\times 40$  in B and C.

occur with single-shock stimuli of high intensity, i.e. stimuli giving galvanometric nerve-responses of beyond the "limiting maximal value" (Forbes and

Gregg). For supramaximal contraction given by the mammalian nerve-muscle preparation under a single shock, reference can be made also to F. Bottazzi,

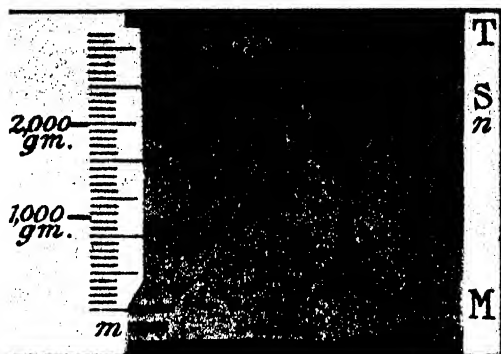


FIG. 2. *Tibialis anticus*: motor-nerve tetanus; break-shocks at 90 per sec.; lettering as in Fig. 1. Tendon movement  $\times 50$ .

*Le contrazioni del preparato diaphragmatico provocate da stimoli unici*, 'Rend. R. Acc. d. Lincei' (5), vol. XXIV, 1<sup>o</sup> Sem., p. 172, 1915.

### I.—Spinal Reflexes.

#### (1) *Ipsilateral Flexion Reflex.*

A. *Semitendinosus*: afferent nerve, peroneal-popliteal. The reflex tetanus shows very evident separate tension waves of 50 per sec. rhythm to stimulation of the afferent nerve by break-shock series of that rhythm. The incompleteness of the fusion is sometimes quite as marked as in the mn. tetani under stimulation at the same rhythm as employed for the reflex (fig. 3), but sometimes it is not so marked as in the mn. tetani. Also with stimulation-frequency at 72 per sec., contraction waves at that frequency have been unmistakable in the reflex tetanus. At a stimulation frequency of 36 per sec. the removal of one stimulus (by short circuit of the secondary circuit for  $\frac{1}{10}$  sec.) during the ascent of the reflex tetanus curve causes a break in the ascent, owing to the lapse of one step from the series of the ascent. It would be so also doubtless with higher stimulus frequencies but we have not extended the observation further.

B. *Tibialis anticus*: afferent nerve, popliteal. Individual contraction-tensions of 50 per sec. rhythm corresponding with break-shock (also with make-shock)\* series applied to the afferent nerve at 50 per sec. frequency are about

\* This extends a previous observation which traced mechanical rhythm corresponding with the stimulus frequency up to 68 per sec. in the isometric record of the reflex tetanus



as marked (Plate 7, fig. 4) in the reflex tetanus as in the case of semitendinosus, and sometimes quite as marked as in the mn. tetani from the same preparation as

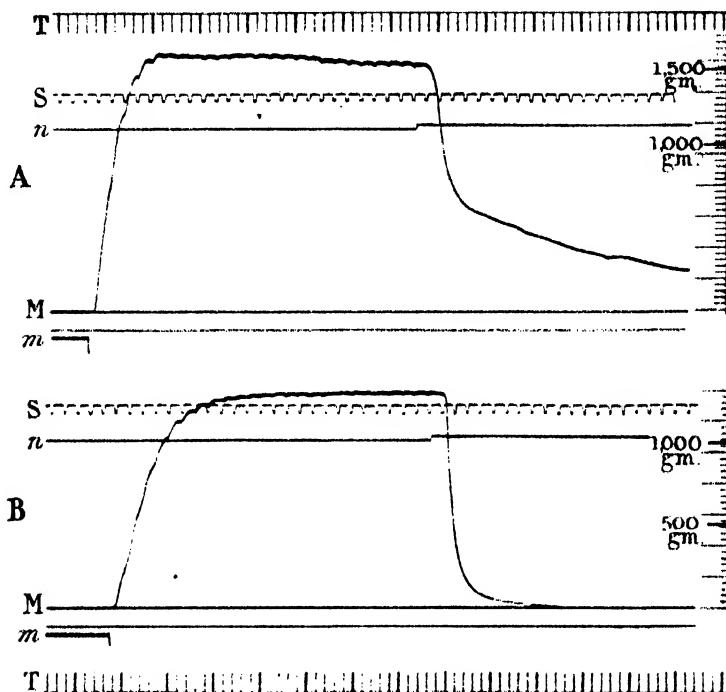


FIG. 3. *Semitendinosus*: A, reflex tetanus, spinal preparation, afferent nerve ipsilateral peroneo-popliteal: B, motor-nerve tetanus, in same preparation as yielded A; in both break-shocks at 38 per sec. T, in 1/50 sec.; other lettering as in previous figs. Tendon movement  $\times 35$ .

yield the reflex. Also when the reflex tetanus has been evoked by 90 break-shocks per sec. to the afferent nerve, separate tension waves of 90 per sec. are sometimes just perceptible in the record; they are best seen near the top of the ascent where the ascent curve joins the plateau. The excision (by appropriate short-circuiting of the secondary circuit) of a single stimulus from a series at 50 per sec. documents itself (fig. 5) by a notch in the reflex tetanus plateau, very much as does similar excision in the mn. tetanus. The steepness of the fall in the notch in the reflex tetanus depends not only on the height of the tension plateau but on the amount and character of the after-discharge of the reflex; the re-ascent from the fall requires in the reflex, as in the mn. tetanus, of this muscle and reflex, but in that observation the stimuli were double shocks of that rhythm, and it was not possible to know whether the make-shocks as well as the break-shocks were effective on the afferent nerve. Action-current rhythmic response has been traced to much higher frequencies (*cf.* E. D. Adrian, 'Journ. of Physiology,' 1923).

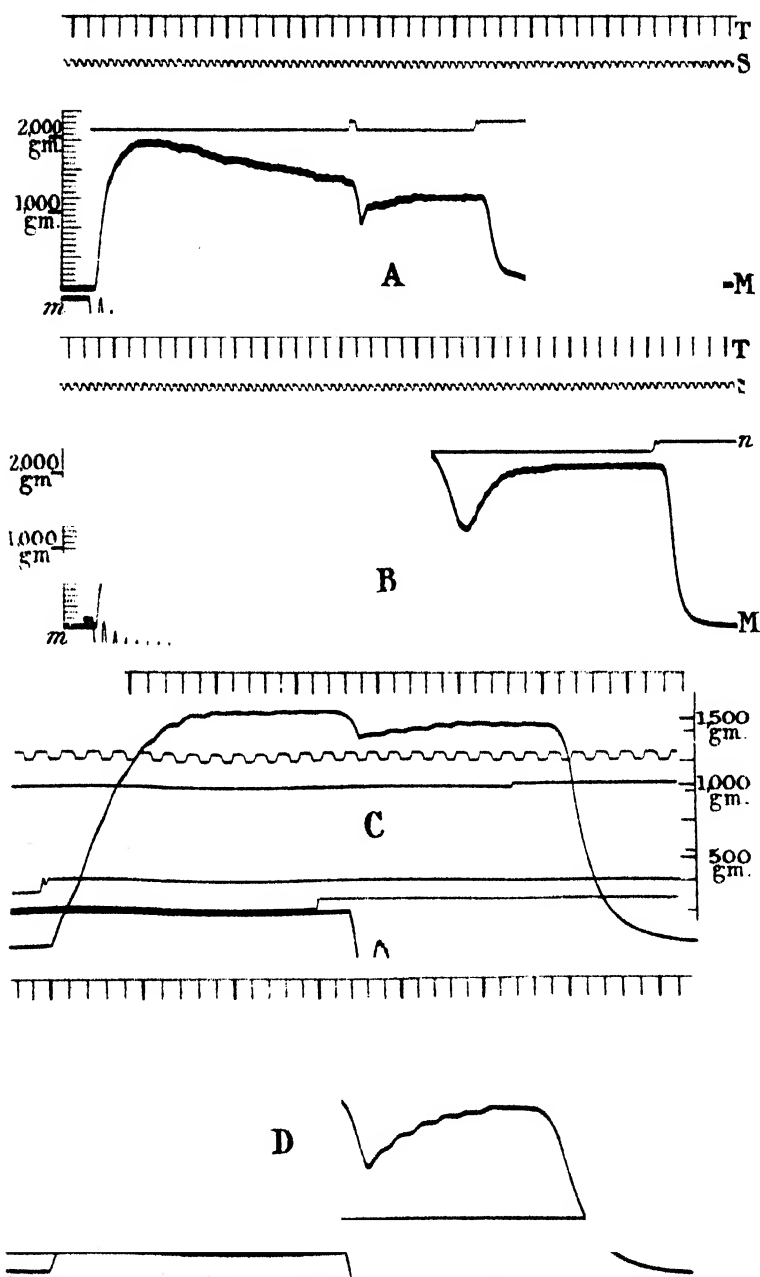


FIG. 5. *Tibialis anticus*: A, reflex tetanus, spinal preparation; excision of one stimulus by short-circuit for  $1/50$  sec., see n. line; B, mn. tetanus; in both break-shocks at 50 per sec.; in B excision of four successive stimuli by short-circuit for  $4/50$  sec. Other lettering as before. C, mn. tetanus, *Semitendinosus*: excision of one stimulus; D, excision of two stimuli; stim. at 39 per sec. Tendon movement  $\times 50$  in A and B;  $\times 35$  in C.

more than one stimulus to rebuild the tension lost by one stimulus excised; nor is the previous tension usually fully regained, either in the mn. tetanus or in the reflex. The break in the tension plateau height is, of course, greater where the break in the serial stimuli extends to several stimuli instead of one only (fig. 5). The inability to recover fully the contractile tension previously obtaining seems to rest on some peripheral condition, for it is marked in the mn. tetanus. The contraction steps in the re-ascent from the fall are smaller than the contraction steps at the similar niveau in the original tetanic ascent to the plateau. The phenomenon is not confined to tibialis anticus: it is seen equally with semitendinosus. It seems referable to the muscle-fibres themselves, since it is difficult to account for it by yield of the muscle's connective tissue.

With stimulus-series of similar frequency, the same incompleteness of fusion of the individual reflex contraction waves met with under stimulation of the peroneal-popliteal as afferent is met with also when the reflex tetanus is provoked from internal saphenous or the femoral nerve. So also when the digital skin (Plate 7, fig. 6) is similarly stimulated, *e.g.* by unipolar faradisation by a stigmatic electrode (entomological pin) inserted less than 1 mm. deep into the skin, the anode being a diffuse saline-soaked pad under a copper plate applied to one of the forelimbs.

Nor is this character of the reflex tetanus altered when the spinal section instead of being performed an hour or so before recording the reflexes is made a week or so earlier. Nor does severance of the proprioceptive afferent nerve fibres of the muscle itself either immediately or several weeks prior to the examination of the reflex obviously alter this character of the reflex tetani.

(2) Ipsilateral extensor reflex. *Quadriceps femoris* muscle with peroneo-popliteal nerve as afferent. The reflex contraction is weak even under strong stimuli, and has not lent itself to satisfactory analysis in the above respect by our methods.

## II.—Decerebrate Reflexes.

### A. Ipsilateral.

(1) Flexor reflex: A. *Semitendinosus* muscle: afferent nerve, peroneo-popliteal. Tremor of the same rate as the stimulus has been very obvious with stimulus frequencies of 35 and 45 per sec., and still marked at 50 per sec. (fig. 7), about as marked as in the mn. tetanus from the same preparation at the same stimulus frequency. This reflex is readily subjected to reflex inhibition by stimulation of *e.g.* any afferent nerve of the contralateral hind limb. During

such inhibition, of degree sufficient to reduce but not to extinguish the reflex contraction, the reflex tetanus continues to exhibit both in the descent and in

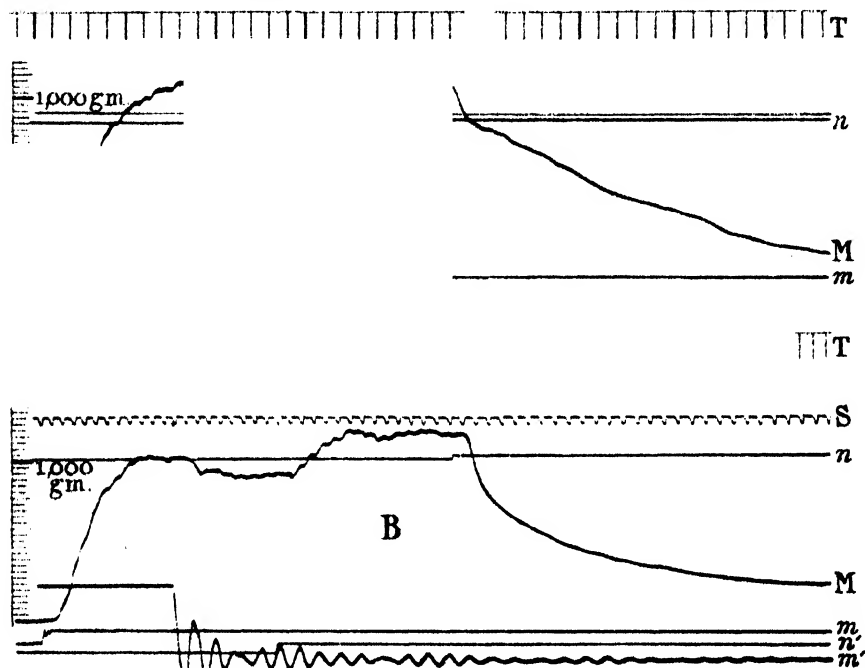


FIG. 7. *Semilendinosus*, decerebrate preparation; reflex tetanus; afferent nerve, ipsilateral peroneo-popliteal. In A stimulus rate is 45 per sec.; in B is 40 per sec. In B intercurrent faradisation of contralateral peroneo-popliteal, see signals  $m'$  and  $n'$ , giving partial inhibition followed by rebound augmentation; the contraction rhythm persists during, and is augmented after, the reflex inhibition. T in 1/25 sec. in A; in 1/50 sec. in B. Tendon movement  $\times 35$ .

the floor of the inhibitory trough rhythmic contraction-waves corresponding with the stimulus frequency. This suggests that under the partial inhibition the reflex activation of a certain percentage of the involved muscle-fibres—and therefore, since the inhibition is central, of the moto-neurones of the centre—is suppressed, while that of the remainder continues seemingly unimpaired. The observations, in fact, support the suggestion made by Keith Lucas and Adrian ("Conduction of the Nervous Impulse," p. 99, London, 1917) and also, with experimental support of other kind, by A. Forbes ('Physiol. Review,' II, 391; July, 1922) regarding the mode of operation of concurrent reflex excitation and inhibition in producing algebraic summation of effect.

**B. *Tibialis anticus* muscle:** afferent nerve, popliteal. Stimulation at 50 per sec. gives reflex tetani in which in some instances tension waves of

corresponding rhythm are obvious; but they are not so obvious as in semi-tendinosus, and may be quite faint even at 38 per sec. rhythm. They are not so obvious as in the spinal preparation. When they are obvious the terminal relaxation of the tetanus is quick in the upper part of its descent, but in the decerebrate reflex that form of descent does not always occur.

(2) Extensor reflex. *Vasto-crureus muscle*: afferent nerve, peroneo-popliteal. This interesting reflex,\* never developing so high a plateau as can the foregoing, and therefore not lending itself so well as they to the detection of individual tension waves, reveals quite perceptible rhythm corresponding with the stimulus frequency even at 90 per sec.

#### B. Contralateral.

*Quadriceps femoris muscle*: afferent nerve, the peroneo-popliteal of the contralateral limb. There is no evidence of separate contraction waves in the isometric records at or above 40 per sec. stimulus frequency, nor is there usually at 35 per sec. (fig. 8), but at this last rate slight indications of waves at corresponding rhythm are sometimes detectable. With stimulation of the afferent

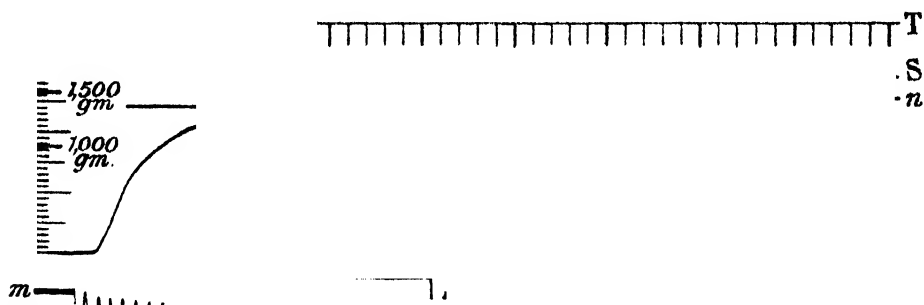


FIG. 8. *Vasto-crureus*: decerebrate preparation; reflex tetanus; afferent nerve, contralateral peroneo-popliteal; break-shocks at 35 per sec. Lettering as before. T in  $1/25$  sec. Tendon movement  $\times 35$ .

nerve by break-shock series at 49 per sec. rate the lapse of three consecutive stimuli (fig. 9) does not impair the smoothness of the plateau level nor even check the smooth rise of the ascent of the tetanus. But the excision of four consecutive stimuli causes an obvious check (fig. 9) in the ascent, although hardly any actual decline of tension. In a few out of several experiments in which the muscle, either at the time of the experiment or some weeks previously, had been de-afferented there have been distinct traces of stimulus-rhythm in the tetani

\* Sherrington and Sowton, these 'Proceedings,' B, Vol. LXXXIII, p. 535, 1911; T. Graham Brown and Sherrington, 'Journ. of Physiol.,' Vol. XLIV, p. 125, 1912.

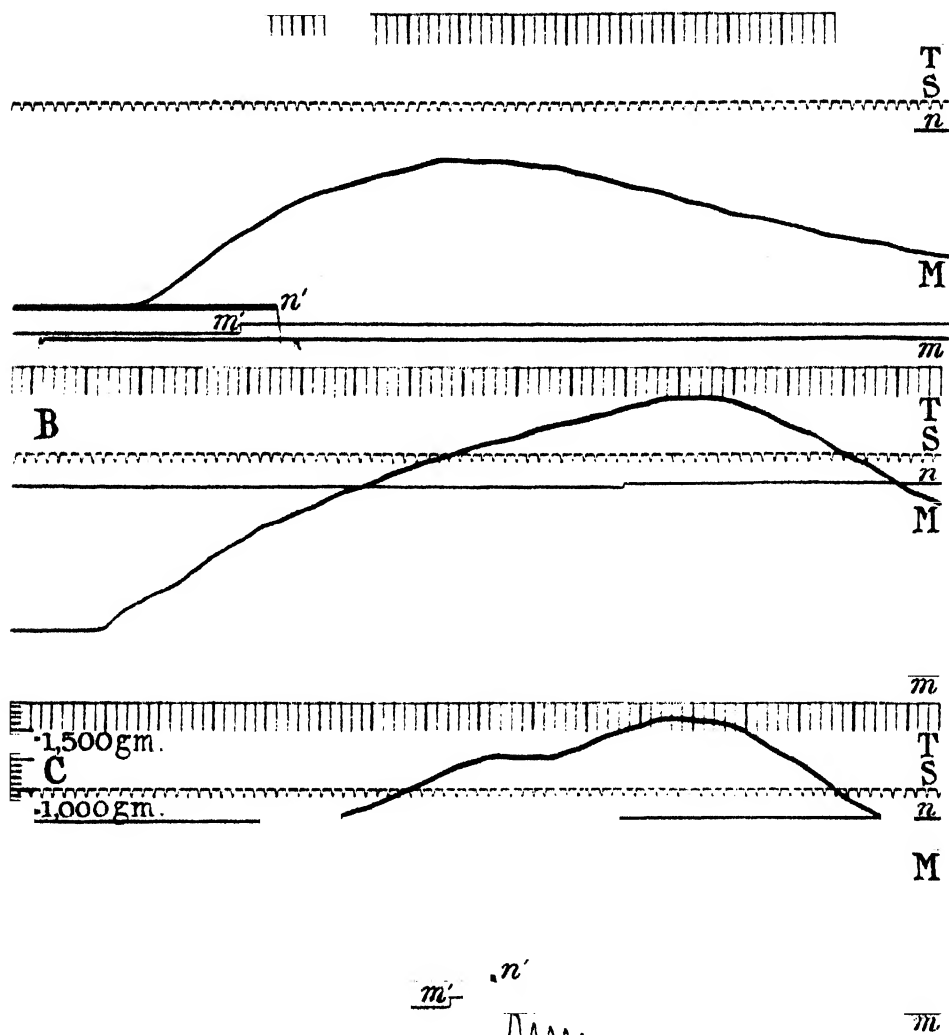


FIG. 9. *Vasto-crureus*: decerebrate preparation, reflex tetanus, afferent nerve contra-lateral peroneo-popliteal; break-shocks, 48 per sec. In A, lapse of three successive stimuli between marks given by short-circuit signals  $m'$  and  $n'$ . In C, lapse of four (? five) successive stimuli. B, stimulation the same as in C, but without any lapse. The tension scale applies to A and B as well as C. Lettering as before. T, 1/50 sec. Stimulus intensity in A, 15 cm. secondary coil, primary (coreless) 0.06 amp.; in B and C similar to A, but with secondary coil at 16 cm.; threshold, 16.5 cm. Tendon movement  $\times 35$ .

evoked by a stimulus frequency of 38 per sec. Intercurrent partial inhibition tends to bring out the stimulus-rhythm of the excitatory reflex (fig. 10) both in the descent and re-ascent of the inhibitory notch cut into the reflex tetanus,

even when that inhibition is induced by a single break-shock to the ipsilateral nerve, and therefore when the inhibitory reflex itself cannot exhibit any

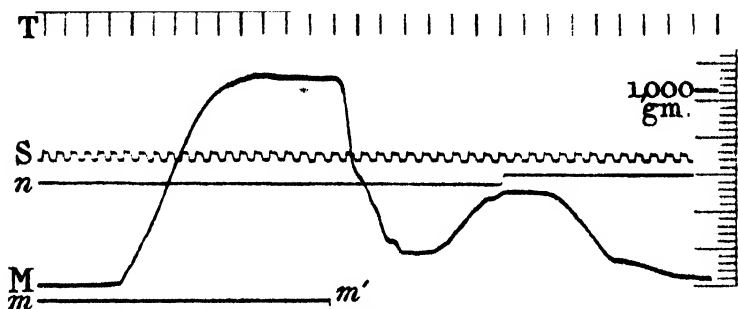


FIG. 10. *Vasto-crureus*, decerebrate preparation; reflex tetanus, afferent nerve, contralateral peroneo-popliteal; break-shocks, 38 per sec. Intercurrent stimulation of ipsilateral peroneo-popliteal by a single break-shock, signal  $m'$ . Time,  $1/25$  sec. Tendon movement  $\times 40$ .

stimulus rhythm at all corresponding with that of the excitatory stimulus, *e.g.* 38 per sec. This, like observations mentioned above (II A (1) A), suggests that the inhibition is partial because it suppresses the activation of a fractional percentage only of the moto-neurons concerned in the excitatory reflex.

*Intercurrent Reflex inhibition contrasted with lapse of excitation of excitatory afferent nerve.*—It might be thought that reflex inhibition interrupting a reflex tetanus would be equivalent in its effect to a correspondingly timed simple lapse of centripetal impulses arriving at the centre from the stimulated afferent nerve. That reflex inhibition is in fact something different from such mere interruption of the centripetal impulses of the afferent nerve is evidenced by several features of its effect. We instance two from the observations here under consideration. In fig. 7B, taken from the decerebrate crossed reflex of semitendinosus, the inhibitory stimulus was given for a period corresponding with eight successive stimuli of the excitatory afferent nerve, and the plateau tension of the reflex tetanus fell to a lower degree. Now, after a simple lapse of stimuli in the reflex tetanus, as also in the mn. tetanus, the re-ascent from the trough shows, as instanced by figs. 4A and 4B, smaller upward steps than at corresponding levels of the original ascent of the tetanus. Whereas (fig. 7B) the recovery from the inhibitory trough after cessation of the inhibitory stimulus is by larger steps than those of corresponding niveaux in the original ascent, and the original plateau level is not only regained but surpassed.

Again, in *Vasto-crureus*, in Plate 8, fig. 11, the effect of lapse of application to the excitatory afferent nerve of six consecutive stimuli from a break-shock series

at 49 per sec. is compared with stimulation of the inhibitory afferent nerve for a period whose duration was intended to cover similarly the delivery of five excitatory stimuli, but in fact extended to the period of three only. The effect of the simple lapse of the six excitatory stimuli, which, being a complete lapse, must mean entire desistence of all centripetal impulses and therefore must affect not some but all of the moto-neurones, is a shallow notch of 100 gm. in the tension plateau of 1900 gm. The effect, on the other hand, of the reflex inhibition operative during the period of but three excitatory stimuli was to reduce the tension plateau of 1900 gm. by 1400 gm. in 0.06 sec. and during the next 0.08 sec. by a further 200 gm., and this in face of unintermitted stimulation of the excitatory nerve; and though unintermitted stimulation continued during a further 0.5 sec., the contraction tension still remained 300 gm. below its original value, although the inhibitory stimulus lasted but 0.085 sec. in all. Fig. 10 contrasts the effect of the lapse of eight consecutive stimuli of the afferent excitatory nerve with that of stimulation of the afferent inhibitory nerve for a corresponding period. The lapse of the eight stimuli causes the tension to fall from 2000 gm. to a little over 1800 gm.; and the relaxation is fully recovered from in 0.35 sec. The reflex inhibition stimulus of like duration reduces in 0.1 sec. the pull from 1800 gm. to 100 gm., and in another 0.15 sec. has abolished it altogether; and the continuance of the excitatory stimulus during the next half sec. only restored the pull to 75 per cent. of what it had been prior to the inhibition. Evidently the inhibition thrusts itself into the excited reflex arc at some central mechanism beyond the seat of momentum, much further downstream in the arc than are the terminals of the fibres of the excitatory afferent nerve. It is noteworthy that the speed of decline of the muscle tension under the inhibitory relaxation is of the same order as that of the relaxation of the mn. tetanus on cessation of the faradisation of the motor nerve itself (*cf.* figs. 1c, vasto-crureus with figs. 11 and 12, Plates 8 and 9).

As regards the differences between the various type reflexes in respect of the tendency of their tetani to resonate to the stimulus frequency of the excitatory afferent nerve, it is in the behaviour of the knee-extensor, quadriceps femoris, in its decerebrate crossed reflex, that the outstanding divergence lies. In the other reflexes, which are all ipsilateral, the fusion-frequency approximates to that of the tetani evoked by direct stimulation of the motor nerve itself. Of the factors which lead to this smothering of the stimulus rhythm at such relatively low frequencies in the decerebrate crossed reflex of quadriceps one would appear to be the decerebrate condition itself, for the discrete character of the individual reflex contraction-waves in the ipsilateral flexion reflex tends in our experience,



although with exceptions, to be less marked in the decerebrate than in the spinal preparation. Comparison with a crossed reflex of the knee-extensor in the spinal condition has failed us for we have rarely succeeded in evoking such a reflex. The cause of the smothering of the stimulus rhythm cannot attach to the afferent nerves fibres, for they presumably are the same in the case of all the reflexes examined. Neither does it lie in the motor nerve fibres and muscle fibres of the quadriceps itself; the character of the *mn. tetani* establishes that. The cause must therefore lie in the inclusion within the central path of the decerebrate crossed extensor reflex of some mechanism other than, and probably additional to, those included in the central path of the other reflexes examined. There is in the crossed decerebrate reflex of quadriceps extensor, so to say, a momentum of reaction—illustrated also by the prolonged after-discharge characteristic of this reflex—which tends to obliterate the stimulus rhythm, and even coarse fluctuations in that rhythm. But comparison of the effect of transient intermission of such stimuli with that of transient reflex inhibition shows that the latter not only renders impotent the centripetal impulses, but unlike the former, controls and can suppress the central momentum of the reflex.

## APPENDIX.

By Sir CHARLES SHERRINGTON.

### *Separation Key.*

Where it is desirable to have a single (kathodal) seat of stimulation and to know the number and rate of delivery of stimuli that may from series to series be varied freely in intensity, although equable within each series, it is advantageous to separate the break-shocks from the make-shocks of the inductorium, so as to apply series of either apart from the other, thus avoiding ambiguities accompanying the use of double shocks. With frequencies up to 220 per sec. a convenient device for thus separating them is the following:—

### *Key for separating break-shock from make-shocks.*

A steel wire A stretched horizontally carries at its mid-length a light horizontal iron cross-piece bearing at each end a fine vertical needle. The cross-piece forms the armature for a small electro-magnet with one pole over one free end of the cross-piece, the other pole below the opposite end of the cross-piece. Below the needle at each end of the cross-piece is an ebonite cup containing a pool of mercury. Lengthwise under the horizontal wire runs a slotted brass bar

carrying two strong clamps, one on either side of the mid-point of the wire. By these clamps the wire can be held firmly at any desired two points equidistant from the mid-point of its length.

If the horizontal cross-piece be inclined by pushing up one of its free ends, it torses the wire to which it is fixed. On release, under the elasticity of the wire, it performs a number of pendular vibrations about the long axis of the wire. The period of these can be varied through a considerable range by varying the length of the wire between the adjustable clamps.

Two similar wires, B and C, similarly furnished with cross-pieces armed with end-needles and similarly arranged for, electro-magnets and mercury pools, and with adjustable clamps, are installed on the same wooden base as A. By means of their adjustable clamps, B and C can be readily tuned so as to have with their cross-pieces, under torsion, the same vibrational frequency as A.

Of the two vertical needles, one at each end of the cross-piece armature of wire A, one ( $\alpha$ ) is in metallic contact through the armature with wire A. When  $\alpha$  dips in the mercury pool below it a current runs through the electro-magnet of A and tilts the armature so that needle  $\alpha$  is withdrawn and the current broken. The wire is thus set into torsional vibration and breaks and makes the current with a frequency depending on the period of the torsional vibration of itself as loaded with its cross-piece. Into this electric circuit is introduced the electro-magnet of one of the other wires, *e.g.* B. The wire B and its cross-piece thus vibrate torsionally about B's long axis, and by arranging a circuit through it and one ( $\beta$ ) of its cross-piece's end-needles and the mercury pool into which the needle dips periodically under the torsion, a current in that circuit is made and broken with the same frequency and practically synchronously with  $\alpha$ 's current.

The needle ( $\gamma$ ) at the end of A's cross-piece opposite to that carrying needle  $\alpha$  is insulated from A and its cross-piece and forms part of a circuit which runs through the electro-magnet of which wire C's cross-piece is the armature; this circuit is completed when needle  $\gamma$  dips into the mercury pool below it. In this way wire C and its cross-piece is set into torsional vibration of the same frequency as A. One of the needles carried by C's cross-piece on dipping into the mercury pool below it completes a circuit running through wire C and its cross-piece. Thus are obtained two torsional wire-keys, B and C, making and breaking two separate circuits with the same frequency as and synchronously with the torsion wire-key A, and therefore with the same frequency and synchronously with each other. If the level of the mercury in one of the cups under wire A's cross-piece be then lowered and that in the cup under the other end of the cross-

piece raised, the torsional vibration in the wire-keys B and C remains of course of the same frequency as before, but they are no longer synchronous in phase. If the discrepancy of phase be set at a quarter of a full vibration period, the circuit in B will be closed at the moment when it is broken in C, or *vice versa*. To set the key so as to separate make-shocks from break-shocks all that is required is to adjust the heights of the Hg pools under the contacts belonging to the cross-piece of wire A, so that in one of them the moment of contact with the mercury occurs a quarter of a vibration earlier than does that of the other.

Thus, if the amplitude of the vibration of the end of the cross-piece be 3 mm. the surface of one Hg pool is set 2 mm. above the point of the contact-needle of that end, when the wire is not vibrating, and the surface of the other Hg pool is set 2 mm. below the point of the contact-needle of the other end. The amplitude of the vibration can be controlled by adjusting the distance of the poles of the electro-magnet of which A's cross-piece is the armature. To isolate the make- and break-shocks it is not necessary, of course, that the moments of the two contacts should be exactly a quarter of a vibration apart; a certain range is available. A galvanometer in the secondary circuit shows at once when the separation of the makes from the breaks has been effected. By using B's circuit for the primary coil of an inductorium and C's for the secondary coil, the break-shocks of the inductorium can be led to the stimulating electrodes with exclusion of the make-shocks, these latter being short-circuited, or *vice versa*, the make-shocks with exclusion of the break-shocks.

This device works well; it was demonstrated to the Physiological Society two years ago, and I have used it repeatedly during the last three years. With it it is not difficult to have break-shock (or make-shock) series of 150 per sec. or more. As tests of its performance a record of the separation of the break-shocks and make-shocks from double shocks at 100 per sec. as registered by the String Galvanometer (Cambridge pattern) is given in Plate 10, fig. 13a, and of two weak submaximal tetani of *tibialis anticus* (cat), fig. 13b, on delivery of a break-shock series at 50 per sec. to the motor nerve, since weak submaximal tetani of mammalian nerve-muscle preparations form rather critical test-performances for the equability of an induction-shock series.

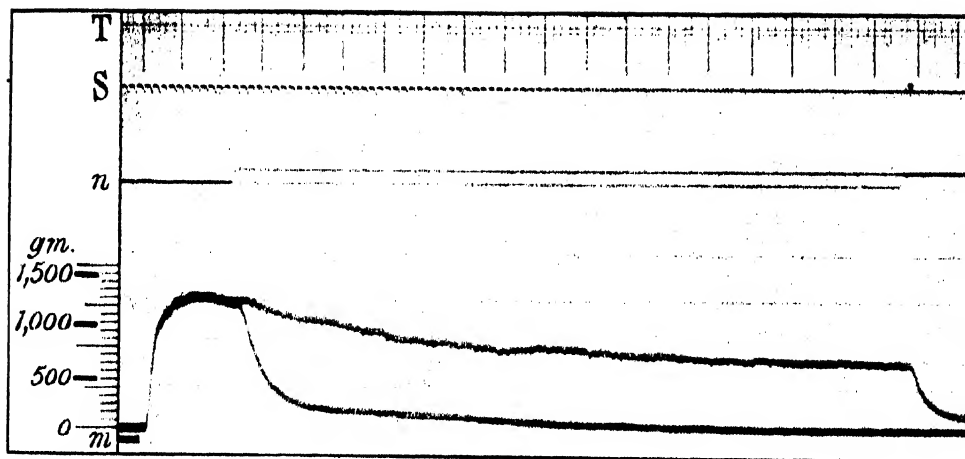


FIG. 4. *Tibialis anticus*: reflex tetanus, spinal preparation, break-shocks at 50 per sec.; afferent nerve ipsilateral popliteal, two successive reflexes, one short and one longer, at same strength of stimulus; other lettering as before. T, in 1/25 sec. T signal, owing to the twofold exposure of the travelling plate for recording the two reflexes in succession, has overrun itself the second time; so also S signal. Tendon movement  $\times 50$ .

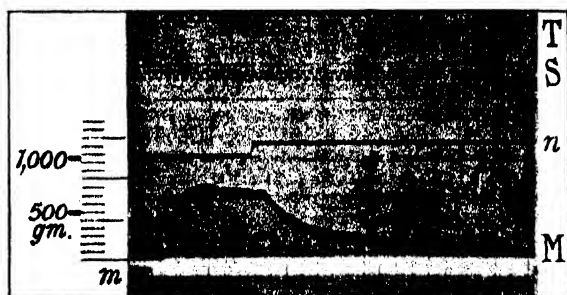


FIG. 6. *Tibialis anticus*, spinal preparation; reflex tetanus by stigmatic unipolar faradisation of skin of digit of ipsilateral foot, 50 break-shocks per sec. Lettering as before. T in 1/25 sec. Tendon movement  $\times 50$ .

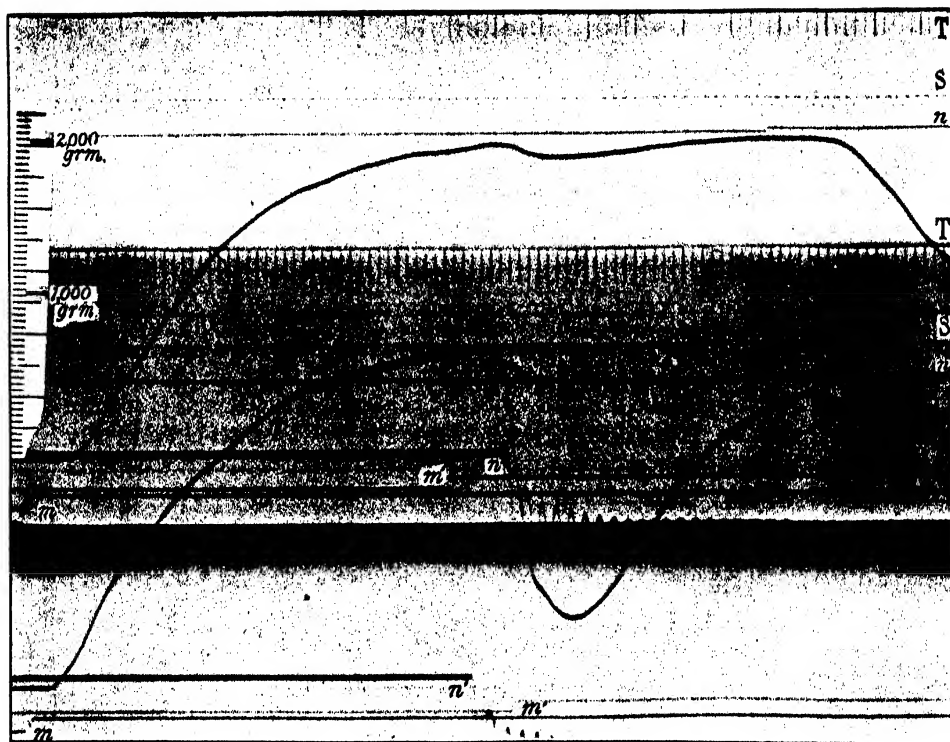


FIG. 11. *Vasto-crureus*, decerebrate: reflex tetanus from crossed peroneo-popliteal nerve by break-shocks 48 per sec. Same intensity of stimuli for both reflexes; in the upper a lapse of six consecutive stimuli during short-circuit marked by signals  $m'$  and  $n'$ ; in the lower no lapse of the stimuli to the excitatory afferent nerve, but an inter-current faradisation of the ipsilateral peroneo-popliteal nerve for the period marked between signals  $n'$  and  $m'$ , a period corresponding to the delivery of three consecutive stimuli to the contralateral afferent nerve. Time,  $1/50$  sec. Same tension scale applicable to both curves; intensity of stimuli for both, 15 cm. secondary coil, primary (coreless), 0.06 amp. Intensity of stimulation to inhibitory nerve, 16 cm. secondary coil; primary coil (coreless), 0.1 amp. in circuit. Tendon movement  $\times 35$ . Lettering as before.

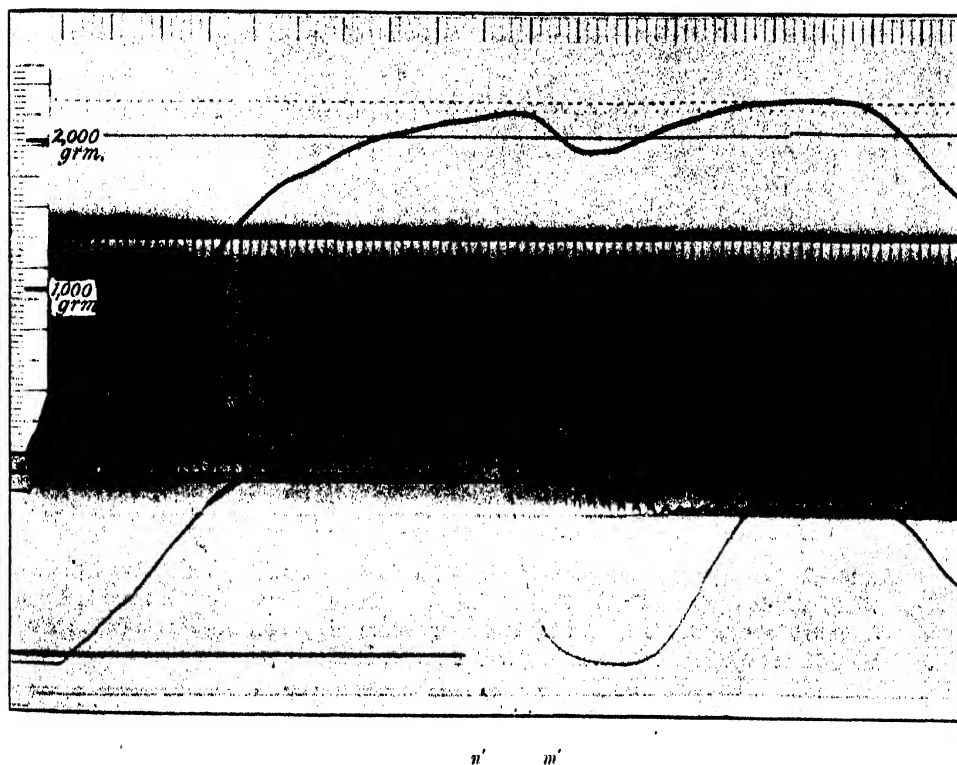


FIG. 12. *Vasto-crureus*, decerebrate: reflex tetanus, as in preceding fig. (fig. 11) but showing result of excision of seven consecutive stimuli in upper curve, and of inhibition for a like period in lower curve between signals  $n'$  and  $m'$ . Lettering, stimulus values, tension scale, etc., as in previous fig. Tendon movement  $\times 35$ .

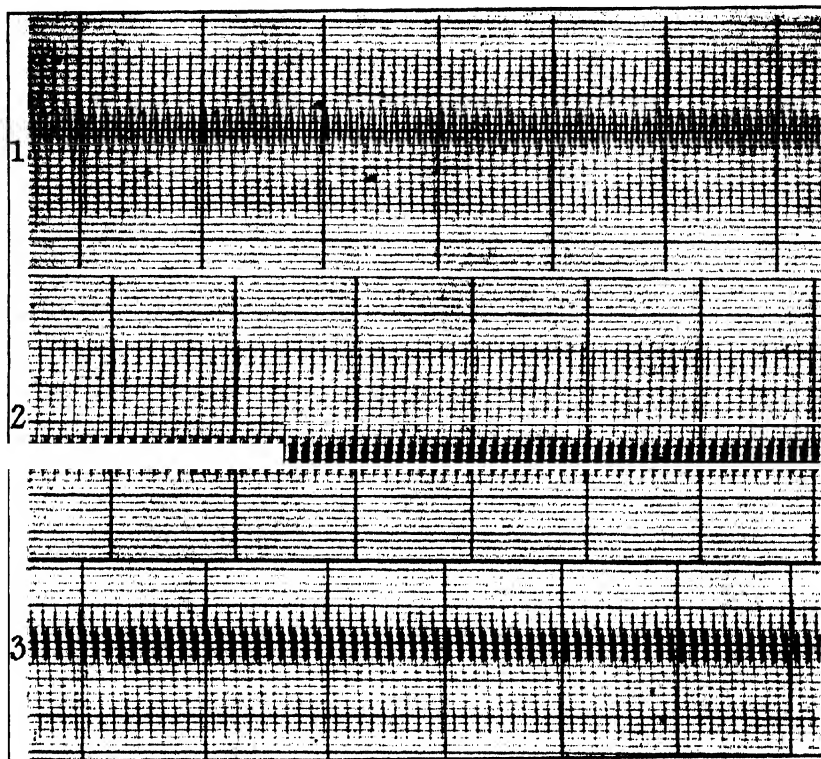


FIG. 13—A. 1. String galvanometer record of induction shocks from ordinary inductorium with torsion-vibration key in primary circuit and giving 100 double shocks per sec. Time divisions in 1/10 sec. 2. Similar, but with torsion-vibration key in primary and secondary circuits, and set so as to allow the break-shocks only to reach the recording galvanometer. 3. Similar, but for make-shocks.

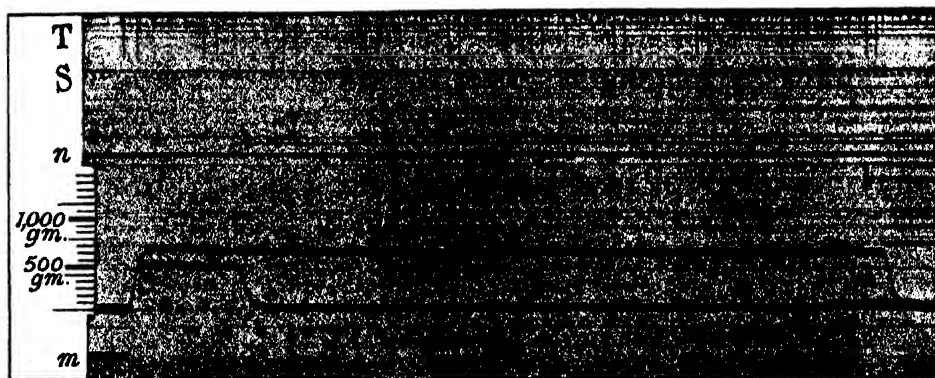


FIG. 13—B. Submaximal mn. tetani (*tibialis anticus*), not far removed from threshold. Tendon movement  $\times 50$ . Lettering as in other figs.







## *The Dusuns of North Borneo.*

By GODFREY HEWETT.

(Communicated by W. B. Hardy, Sec. R.S. Received February 21, 1923.)

The Dusuns, as a race, are the most interesting of all the tribes inhabiting Borneo, and afford much scope for scientific investigation. Their true origin, however, is in danger of being lost, owing to the views expressed by writers of the present century.

In the early days of the Chartered Company it was always accepted as fact that they were the direct descendants of former Chinese settlers. The Dusuns themselves claimed it, and the late Sultan Hashim of Bruni asserted it as beyond question. It was traditional in Bruni, and in this paper an attempt is made to establish the Dusun claim for the sake of record.

The early history of Borneo is not easily grasped. It can only be gathered bit by bit by exceedingly laborious research through the works of innumerable writers, and few will undertake such a task. The key to the whole question of the Dusun claim appears to be in the tradition, that many writers have referred to, that Kublai Khan invaded North Borneo in 1292, but none appear to have treated this seriously, or to have followed it up and applied its conclusions.

In the latest publication, "British North Borneo," by Owen Rutter, the author refers to the tradition, but dismisses it with the regret that Marco Polo did not record it. There were, no doubt, reasons for the omission. Kublai Khan died shortly afterwards in 1294, and Marco Polo returned home (a very long and arduous journey in those days), and was captured in the Mediterranean before reaching home and imprisoned as a prisoner of war for a long time. His great work was written while in prison, and the marvel is that he did not make more omissions.

The tradition is that Kublai Khan invaded North Borneo in great force in 1292 and founded a Chinese Province, in which were included the Sulu Islands. Whilst the tradition is persistent the record is meagre, but fortunately we have ample material from which to weigh the probability of his having done so or not.

When Kublai Khan succeeded to the great Mongol Empire founded by his grandfather, Genghis Khan, it extended from the borders of Poland to the

Persian Gulf, it included Central Asia and all Siberia to the Pacific, but it did not include the Chinese Empire proper, or any of the countries to the South of China. Kublai Khan immediately set to work and succeeded in establishing his authority over China and all the other States down to the Straits of Malacca. His Empire then covered the whole Continent of Asia, with the exception of India.

In China he found the richest asset of his whole dominions. China was the one great maritime power of Asia, and her overseas trade was immense and exceedingly lucrative, so much so that the Khan was tempted to continue his conquests beyond the sea. He first sought to bring Japan under his rule, but after the failure of seven or eight expeditions, he abandoned the attempt finally in 1278. It was at this period that Marco Polo was his great friend and associate, and for some years was in the Khan's service, who sent him on a voyage through the Archipelago to report. Marsden refers to his visit to Sumatra in 1290, and on his return to China in 1291 he drew attention to the great and lucrative trade carried on in junks between China and the Islands. China was without a rival afloat, and even the carrying trade between India and the Hindu Empire of Majapahit was largely conducted in Chinese junks. China traded as far as the African coast and even extorted tribute from Madagascar.

Now at this time the Hindu Empire of Majapahit, which embraced the Islands of Sumatra, Java, Bali and Lombok, was at the zenith of its power and had overrun a large portion of Western Borneo, including Bruni and Sarawak, where many thousands of Chinese had long been settled, and made Bruni into a tributary State.

It is probable that, for this reason, Kublai Khan sent an envoy, as was his custom, to demand submission, homage and tribute from Majapahit. His envoy was sent back mutilated with an insulting message, and the Khan immediately sent a great expedition against Majapahit which landed in Java, but was compelled to re-embark with the loss of 3,000 men. It is not recorded that any further attempt was made to reduce Majapahit by force of arms.

Before reading further let anyone take a look at the map of Asia and the Eastern Archipelago, and especially note the position occupied by North Borneo, and bear in mind that this Chinese trade had been carried on unchecked in all probability for at least 2,000 years. Not only was there great commercial intercourse between China and Majapahit, but China had a monopoly of the trade with all the rest of the Archipelago, and this entailed the settlement

of many thousands of Chinese throughout the Islands, to conduct the trade operations and furnish produce and material by industrial enterprise such as mining and planting.

To protect this trade, the most valuable of all the Khan's interests, it was necessary to interpose a check to the further extension of Majapahit, and the obvious move was the acquisition of North Borneo, Bruni being the furthest limit of Majapahit's extension.

Now, how did the tradition arise that the Khan invaded North Borneo in 1292, immediately after the repulse in Java, if he did not do so? The Khan was the last man to sit down under a repulse and do nothing. There had been great intercourse between China and Bruni for a long period previous to this, and the invasion must be accepted as fact if only to account for what we know to have followed. There was increased intercourse, and closer relations were established with Bruni, until the throne of Bruni actually came into Chinese hands, and the tribute to Majapahit by Bruni was stopped and diverted to China. The invasion of 1292 must have been followed by a period of settlement and development, and there is plenty of evidence that this was the case, and that large numbers of Chinese came to the country, not only to the Chinese Province of North Borneo, but to Bruni territory as well. St. John, in his "Life in the Forests of the Far East," records how the Limbang River had been thickly populated with Chinese to a distance of 180 miles from the mouth.

In North Borneo, in country occupied by the Dusuns, on the West there are numerous irrigation channels still in use that could not have been constructed except by a highly experienced designer with scientific knowledge. These occur all down the Tambunan Valley, and there is one very long one at Ranau, which lies South-East of Kinabalu. On the coast, where the mountains descend abruptly into flat alluvial plains, no leats are necessary, and the practice of tapping a stream at the foot of the hills and retaining the water by mud embankments round the fields is scientific simplicity itself. The Chinese, with their great experience, are the only people who could have carried out all these works.

Another, but smaller, piece of evidence is the bamboo bridge over the Tampasuk River at Kaung Ulu, a survival of Chinese days. No Dusun nowadays could design and construct such a bridge. It is a compromise, or, rather, a combination of the cantilever and suspension principles, and there are many of this and other types in the interior of China, and notably in Thibet and the Shan States.

We learn also from Sulu records that Ong Sum Ping settled in the Kinabatangan River in 1375, and in the "Selesilah" of Bruni, the Book of Descent of the Mahomedan Sultans of that State, he is described as the "Chinese Rajah of Kinabatangan," and his daughter married Sultan Akhmed, the second Sultan, and it was on this alliance that the Bruni Government based their claim to the whole territory of North Borneo when negotiating with Mr. Alfred Dent, as he then was, and Baron Overbeck. It is not improbable that Ong Sum Ping was the occupant of the Bruni throne in the beginning of the 15th century, referred to by Groeneveldt in his "Essays relating to Indo-China."

The Dusuns themselves claim descent from Chinese, and this claim cannot be disregarded because they have varied in type, customs, or language since they were abandoned by their own Government some 200 years ago.

The wide distribution of the Dusuns throughout North Borneo shows that the country was thoroughly occupied and settled, and there is every reason to believe that the occupation lasted some 400 years. We must bear in mind that the original object of Kublai Khan was the protection of the trade of China, and the occupation continued until the trade was deliberately destroyed and stolen by the Dutch. The records of how and when this was done are very full and complete, and need not be gone into here.

Even the name of the great mountain Kinabalu, the Chinese Widow, contributes its small quota of evidence. The name is Malay, and without doubt was given by the Bruni Malays when the Chinese Government abandoned their Colony. It would be typical of the whimsical Bruni humour.

Finally, there is the legend of the Dragon and Gomala to be examined. Nearly all writers refer to it, and all apparently treat it as something meaningless and of no value. There have been a great many versions of this legend, the natural consequence of its being handed down by word of mouth through native channels for something like 150 years. It was written towards the end of the 18th century and preserved in the archives of the Bruni Government, and I regret that I have no copy of the original text. The British North Borneo Company have very kindly supplied me with two versions from the *North Borneo Herald*, widely differing from each other. Various writers have given others, no two of which agree, and I have heard many versions from natives. But through most of the varying versions the essential points are retained, viz.: the Dragon on the top of Kinabalu guarding a fabulous precious jewel called a Gomala, the stealing of the jewel (in some versions by

white men), and the disappearance of the Dragon, leaving certain Chinese behind, from whom the Dusuns claim descent.

This legend is obviously an allegory, and a very clever one. Until the present century, when China became a Republic, the Dragon was the symbol of China, just as the Rising Sun is that of Japan. The Dragon of the allegory meant the Chinese Province in North Borneo guarding the precious jewel, the rich trade of China. The stealing of the jewel referred to the action of the Dutch, and the disappearance of the Dragon to the withdrawal of the Chinese Government and the abandonment of the Province. The Chinese left behind were the settlers from whom the Dusuns claim descent.

There is a rather curious corroboration of this by Earl, who says: "The Chinese suppose the Dyaks to be descended from a large body of their countrymen *left by accident on the Island*, but this opinion is entertained solely on the faith of a Chinese legend. If they can prove their paternity to the Dyaks they must extend it to the whole race inhabiting the interior of the large islands of the Archipelago."

This is a very interesting statement, and, if well founded, a very important one, but it contains a misconception which will take a good deal of disentangling. Earl has evidently been misled by the word Dyak, and before we can see light the name Dyak must be closely investigated. At the present time the only people commonly known as Dyak are those of Sarawak, who call themselves Orang Daya. Dyak is a white man's corruption of this. The word Daya is Bruni Malay and means land, or inland, or land as distinguished from sea. In Sarawak the word Dyak has been further complicated by dividing the Dyaks into Land Dyaks and Sea Dyaks, to meet the white man's requirements; but these people have no Chinese strain in them.

Bampfylde, than whom there is no greater authority on the natives of Sarawak, says:—"The Sea Dyaks are proto-Malays, that is to say, they belong to the same ethnic family, but represent that stock in a purer, less mixed stage. They migrated from the West, probably from Sumatra, at a period previous to the conversion of the Malays to Islam, for their language, which, with slight dialectic differences, is purely Malay, contains no Arabic except of very recent introduction."

That excludes the Sea Dyaks from Earl's statement. The Land Dyaks are an entirely different race with a different language, and are regarded as of Hindu origin, and that relieves them of any Chinese claim to paternity.

In the search for the Dyaks of whom the Chinese claim the paternity, we

have to fall back on the Dusuns as the only other people to whom the name Dyak has been applied. During a great part of last century the Dusuns were known to the Malays as Dusun Daya, which was corrupted by white men into Sun Dyaks, a name that has puzzled many people. The name Dusun Daya was well known in the early days of the Chartered Company, but appears to have been allowed to lapse. Now, seeing that the Dusun Daya claim descent from the Chinese, and the Chinese suppose the Dyaks, or Daya, to be descended from "a large body of their countrymen left by accident on the Island," it seems conclusive that the Dusuns and Chinese mutually claim kin to each other. Earl says the claim rests "solely on the faith of a Chinese legend," evidently referring to the Bruni allegory, which is only Chinese in substance. It seems obvious that the occupation and the allegory hang together, either one compelling acceptance of the other.

Now consider all the foregoing details together, the known conditions in Asia and the further East during the 13th century, the two great Empires, that of India and Majapahit, and the great Mongol Empire of Kublai Khan, both at their zenith, both expanding, and the inevitable collision; the stated occupation of North Borneo by Kublai Khan at the very point of furthest extension by Majapahit; the loss of influence by the latter, and the gain by the former until the throne of Bruni actually came into Chinese hands; the transfer of the Bruni tribute from Majapahit to China; the evidence of settlement by Chinese during an occupation of some 400 years; all the records of the destruction of the Chinese trade by the Dutch, and the consequent withdrawal by the Chinese Government; the Dusuns claiming descent from the Chinese who were left behind, and the support of Earl's statement; and even the name of Kinabalu and the allegory of the Dragon.

We have here a mosaic of history, fact, tradition and deduction in which the pieces all fit in and support one another in a way that cannot be treated as legendary or open to question, unless one is prepared with something more convincing and more authentic to put in its place. It must be obvious that if the foregoing is not in accordance with what actually took place, the allegory of the Dragon and Gomala would never have been written and deemed worthy of preserving in the archives of Bruni.

There are sound reasons for asserting that the Dusuns have not intermarried with other tribes. In the first place they have retained the true Chinese colour. Then there is only one tribe they could have mixed with, and that is the Muruts, all others being Mahomedans. The Muruts, however, are out of the question owing to their conditions of life and habits. The Dusuns are a clean-living

people, they bathe, keep their houses clean, and eat clean, wholesome food. The Muruts do none of these things, and their conditions of life and habits will not bear stating on paper. Even as regards the country occupied by the Dusuns and Muruts there is no overlapping; both keep to themselves.

There can be no reasonable doubt that the Dusun claim to be descended from Chinese ought to be admitted. They are not in any sense an aboriginal race, and their presence in Borneo cannot be accounted for in any other way.

*The Biological Action of Light.—I. The Influence of Temperature.*

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(From the National Institute for Medical Research, Department of Applied Physiology.)

(PLATE 11.)

In a series of experiments carried out by one of us at Montana-Vermala with the co-operation of Dr. B. Hudson, who afforded facilities for the work at the English Sanatorium, the biological action of Alpine winter sunlight was studied under varying temperature conditions. While the treatment of weakly or tubercular children by sunlight and open air has gained high repute, *e.g.*, by Rollier at Leysin, or Gauvain at the Treloar Hospital, the sun treatment of phthisis is generally regarded unfavourably. The children, more or less nude, according to weather, are exposed on open galleries to sun together with a high cooling power of air, a cooling power which, measured by the katha-thermometer, is two, two and a half and even three times that of an ordinary cool room. On the other hand, phthisical adults, *e.g.*, in Switzerland, are exposed clothed in sun-boxes facing south where they are more or less overheated by combination of sun, warm enclosure and absence of cooling wind. Personal experience shows that Alpine winter sun, plus cooling air, have an exhilarating effect on the nude body, and that, while the body-heat production is put up by the cold, with general advantage to health, no sunburn results.



On the other hand, warm sun in a warm shelter produces sunburn and is oppressive and disadvantageous. The advice of an old sea captain was, "expose yourself to the sun in the prow if you want to get nicely browned, you will get sunburnt if sheltered from wind in the waist of the ship." Bayliss\* writes "The great effect of ultra-violet light on the skin is familiar to everyone in the inflammation (erythema soläre) called sunburn, which results in a brown coloration. It may be pointed out that this is not an effect of heat, in fact, it is more liable to occur in cold surroundings, probably owing in part to the fact that the heat of the sun's rays is not noticed and no means taken to protect the skin from their action."

The Arctic sun with low air temperature can produce severe sun burns. The following statement is made by M. de Laroquette†:—"The calorific rays by provoking hyperæmia and sweating render the epidermis more resistant to the action of the chemical rays. The skin is most fragile when it is dry and made bloodless by cold (Coup de soleil des glaciers)."

Dewar‡ was able to kill phosphorescent bacteria, which survived exposure to the temperature of liquid air, by ultra-violet raying at that temperature. Such cold may have an injurious effect which renders the bacteria more sensitive to radiation.

Bang§ found that bacteria may be killed by a certain intensity of arc light when at 45° C. in 30 seconds, while when at 30° it takes 30 minutes to kill them. Thiele and Wolf|| also found that the warming of the culture fluid heightened the bactericidal action of the ultra-violet rays, also of the longer rays in the presence of oxygen. Weisner confirms the accelerating effect of temperature on the bactericidal action of light.¶

M. de Laroquette\*\* says cooling diminished the bactericidal power of the Algerian sun, but attributes this to the hindrance of evaporation, to which he attributes a large part of the sterilising effect of exposure of culture fluids to the sun. He does not appear to have tried the effects of sun and temperature when evaporation was prevented.

\* 'Principles of Gen. Physiol.,' 3rd edit., p. 571 (1920).

† 'Compt. Rend. Acad. Sc.,' vol. 171, p. 128 (1920).

‡ 'Roy. Instit.' (Jan. 21, 1910).

§ Finsen's 'Mitt.,' p. 93 (1901).

|| 'Arch. f. Hyg.,' vol. 60, p. 29 (1907).

¶ 'Arch. f. Hyg.,' vol. 61, p. 1 (1907).

\*\* 'Ann. Institut Pasteur,' April (1918).

Poynter and Moritz\* exposed snail embryos to the ultra-violet rays for a short time at a temperature of 3-4° C., and found the embryos thereafter lived 5 days at 24° C. and only 2 days at either 7° C. or 39° C. They appeared then sensitised by raying in cold water to either cold or warmth. The excitability of Cyclops to ultra-violet rays was found by V. Henri to be independent of temperature.

Turning to biological photo-chemical reactions, we recall the fact that when light falls on a solution containing oxy-hæmoglobin and carbon monoxide hæmoglobin, it changes the position of equilibrium, causing a reduction in the amount of COHb with a corresponding increase of the O<sub>2</sub>Hb (Haldane). Hartridge and Roughton† find the temperature coefficient of this change is 2·5.

C. Warburg‡ investigating the photo-chemical decomposition of carbon dioxide by algæ (*Chlorella*) found a temperature coefficient of 1 with low intensity of light, while with high intensity of light and concentration of carbon dioxide the coefficient of temperature decreased from 4·3 to 1·6 from 5° C. to 32° C. Seedlings do not become green in the light at a low temperature (Sachs). The cave amphibian, *Proteus anguinus*, when exposed to light becomes pigmented better at a warm than at a cold temperature.

Bovie§ found that albumin coagulated at a lower temperature and paramœcia were sensitised to heat, after raying.

E. G. Lundberg determined the power of the ultra-violet rays to destroy complement in blood serum, and found temperature variations between 26° and 46° to have almost no effect on this.

M. J. Vallett|| followed W. H. Roger¶ in exposing to ultra-violet rays glycerine extracts of pulped tissues, such as liver, brain. These are suspended in a solution of bicarbonate of sodium to which methylene blue is added. A thermometer was placed in the suspension and a control made in every case.

The following table shows the effect of temperature.

\* 'Journ. Exper. Zool.,' vol. 37, p. 1 (1923).

† 'Proc. Roy. Soc.,' B, vol. 94, p. 365 (1923).

‡ 'Biol. Zeitsch.,' vol. 100, p. 230 (1919).

§ 'Science,' vol. 37, pp. 24, 273 (1913), 'Journ. Gen. Physiol.,' vol. 1, p. 331.

|| 'Compt. Rend. Acad. Soc.,' vol. 173, p. 1141 (1921).

¶ 'Rev. Méd.,' vol. 38, p. 1 (1921).

Table I.

Temperature.	Time of reduction in minutes.	
	In feeble light.	In the sun.
38	16	11
36	19	12
32	29	16
28	47	23
24	74	32
20	117	46

The solar radiation during the experiments varied from 1.05 to 1.25 gr. cal. per cm.<sup>2</sup> per minute.

From 38 to 28°, the time of reduction is approximately doubled in a drop of 10° C., while from 28 to 20° C. it is doubled in a drop of 8° C.

The above summary shows that there is some evidence, but not very conclusive, in kind as to the effect of temperature on the biological action of ultra-violet rays, to which rays it is generally admitted the destructive action of light is mainly due. The effect of the various rays of the sun spectrum, if taken of equal intensity, depends on whether they are absorbed or not by the living substance, the action depending on absorption and conversion of radiant energy into some other form, *e.g.*, heat or the discharge of electrons and ionisation. Weisner\* claims that the ultra-red rays are no less bactericidal in power than the ultra-violet, but of this we have found no evidence when the heating effect is stopped.

The question of the biological interference of the various rays will be considered in a subsequent paper, some evidence having been found by us† that the red rays delay the lethal action of the short rays.

The effect of temperature on the biological action of light was brought forcibly to mind of one of us on testing the action of the winter sun at Montana in November and December, 1922. Our colleague, Mr. J. E. Barnard, provided L. H. with a quartz spectrograph for investigating by photography the spectrum of the light of the Alpine sun, blue sky and snowfields. In the sun spectrum given by this instrument, long narrow strips of ciliated epithelium, cut from the roof of the mouth of the frog, were placed and exposed with a view to mapping out the lethal rays. It was found after many hours of exposure on successive days at the open window no effect resulted. It was found also

\* *Loc. cit.*

† *Cf. Hess, 'Journ. Am. Med. Assoc.,' vol. 80, p. 687 (1923).*

that erythema of the skin resulted, when areas on the arm or chest were exposed to the mid-day sun, only when the conditions were warm, not in cold air, although the radiation energy of the sun was sufficient to raise the surface temperature of a piece of black fur up to about 50° C., equal to a July sun in England at mid-day with a very gentle breeze. The black fur was held at right angles to the sun and stroked gently to and fro with the bulb of a thermometer until a constant reading was obtained. The temperature of the air was 7–10° C. at the open window about mid-day, against 22° on the July day in England. Dorno has found at Davos that the winter Alpine sun has a maximal calorific radiation not much less than in summer, *e.g.* 1.49 grm. cal. per cm. per minute in May, and 1.35 in December, but is far less powerful in ultra-violet rays. The high winter sun has no rays shorter than 3080 A.U., while the high summer sun may have rays as short as 2970. The rays which particularly have power to pigment the skin are 3020 to 2970.\* It is agreed that the shorter the rays the more rapid for equal intensity is the lethal effect exerted on micro-organisms (E. Hertel, V. Henri).

In a fresh series of experiments, narrow strips of ciliated epithelium were cut from the roof of the mouth of the frog and placed in 0.6 per cent. saline solution. The preparations were exposed at the open window to the Alpine

Table II.—Ciliated epithelium in 0.6 per cent. saline in wax cells.

Date.	Covering.	Result.
25.11.22	Uviol glass	Active after 4½ hours exposure to sun.
	Thin glass	
29.11.22	Uviol glass	Active "after 195 minutes exposure to sun.
	Thin glass	

winter sun in a wax cell on a glass slide without cover, or covered by thin glass or thick uviol glass, the latter being transparent to the ultra-violet rays. In a room facing south, with wide open windows, the conditions were approximately these :—

Black fur = 47° C. Dry bulb = 10° C. Wet bulb = 5° C. Dry katha-thermometer cooling power = 8.

Under these circumstances, and with the preparation under cold saline solution, it was found that the light action was very delayed, and in several

\* Haussner and Vahle, 'Strahlentherapie,' vol. 13, p. 41.

experiments no lethal action could be demonstrated even after some hours of the exposure of the preparation to the sun.

The biological action of the Alpine winter sun on preparations of hay infusoria was next investigated. In these experiments, a drop of water containing hay infusoria, colpodium, paramœcium, etc., was placed in a wax cell and covered with a thin glass coverslip, or thick uviol glass, or thin mica. In some experiments (Table III, Nos. 4-7) the hay infusoria were exposed in the air, by making a "hanging drop" preparation in an uncovered wax cell. The preparations were placed at right angles to the sun, the effect of the radiation being tested by the degree of motility of the organisms.

The following experiments (see Table III) showed that the lethal action of the sun was delayed by the low temperature of the preparations. When the temperature was raised by resting the glass slide carrying the organisms on a rubber bag, the surface temperature of which was 34° C., the lethal light action became apparent and the organisms were immobilised in 45 minutes. The temperature of the hanging-drop preparations was not raised to a lethal point by placing them on the rubber bag, for controls in the shade continued to live. The surface temperature of the preparation, exposed on its upper side as it was to the cool air, must have been considerably below 34° C. Preparations placed on snow survived long exposures to the sun, so did preparations over the cover of which snow water was irrigated in a thin film.

*Observations on the Production of Erythema and Pigmentation on Exposure of the Skin to the Alpine Winter Sun.*

These observations were carried out by placing a rubber sheet over the chest. This sheet had a number of circular holes 1 inch in diameter punched out. The areas of skin so exposed were covered by different media, such as glass, mica, uviol glass, water and copper sulphate; so as to determine the protective powers of these media. In the early series of experiments it was found that a small film of sweat collected under the media used, and to obviate this interference the sheet was raised from the skin level.

A rubber sheet with six holes punched out was attached to the chest, and sun exposure of the skin was effected over the areas so exposed. The areas were covered respectively by glass, uviol glass, water and copper sulphate solution. In one area the dry naked skin was exposed and in another the naked skin kept wet. The exposure was made at an open window of south aspect.

Table III.

No. of Exp.	Date.	Atmospheric Conditions.	Covering.	Experimental Conditions.	Results.
1	4.12.22	Black fur 49° C. ....	Glass .....	Exposed to sun ....	All immobile in 50 minutes.
			Mica .....	Exposed to sun ....	All immobile in 30 minutes.
			Mica with film of cold water.	Exposed to sun ....	All active in 80 minutes
2	5.12.22	Black fur 45° C.; calm sunny day Light clouds at times.	Mica .....	Exposed to sun ....	Slowed in 90 minutes
			Mica .....	Exposed to sun ....	Nearly all immobile in 120 minutes.
			Mica with film of cold water from syphonage.	Exposed to sun ....	All active in 360 minutes.
3	9.12.22	Black fur 51° C., dry bulb 9° C.; dry kata 8.	Glass .....	Resting on snow; exposed to sun.	All active after 100 minutes; slowed after 210 minutes.
			Glass with film of cold water from syphonage.	Exposed to sun ....	All active after 100 minutes; all immobile after 210 minutes.
			Glass .....	Resting on window sill; exposed to sun.	Nearly all immobile after 100 minutes; all immobile after 210 minutes.
			Glass .....	In shade .....	All active after 210 minutes.
4	10.12.22	Black fur 37° C. ....	Glass .....	Exposed to sun; resting on rubber water bottle, 30° C.	All inactive after 130 minutes.
			Glass .....	In shade; resting on rubber water bottle, 30° C.	All active after 145 minutes.
			Glass .....	Resting on window sill; exposed to sun.	Slowed after 130 minutes; many immobile after 145 minutes.
			Glass .....	In shade .....	All active after 145 minutes.

Table III.—contd.

No. of Exp.	Date.	Atmospheric Conditions.	Covering.	Experimental Conditions.	Results.
5	11.12.22	Black fur 49° C.; dry bulb 7° C.	Glass .....	Exposed to sun; resting on rubber water bottle at 34° C.	Immobile after 45 minutes.
			Glass .....	Exposed to sun; resting on snow.	Active after 45 minutes.
			Glass .....	Exposed to sun; resting on white china dish on window sill.	Immobile after 45 minutes.
			Glass .....	Exposed to sun; resting on snow.	Active after 55 minutes.
			Glass .....	Exposed to sun; resting on warm rubber bottle at 34° C.	Many inactive after 55 minutes.
			Glass .....	In shade; resting on warm rubber bottle at 34° C.	All active after 55 minutes.
6	12.12.22	Black fur 50·5° C.; dry bulb 10° C.; wet bulb 5° C.; dry kata 6.	Glass .....	Exposed to sun; resting on rubber water bottle at 33° C.	Slowed after 52 minutes; immobile in 90 minutes.
			Glass .....	In shade; resting on rubber water bottle at 33° C.	All active in 90 minutes.
			Glass .....	Exposed to sun; resting on snow.	Sluggish after 100 minutes, but active after slight warming.
7	14.12.22	Warm sun, calm day.	Glass .....	Exposed to sun; resting on snow.	All active after 240 minutes.

Table IV.

	19.11.22.	21.11.22.	24.11.22.
	1½ hours exposure to sun.	2 hours exposure.	2 hours exposure.
A.—Glass .....	Erythema and Pigmentation (3) = slight.	Slight reaction (3).	Slight reaction (4).
B.—Uviol .....	Marked reaction (2).	Marked reaction (2).	Marked reaction (3).
C.—Copper Sulphate.	No reaction (4).	No reaction (4).	No reaction (5).
D.—Wet .....	Very marked (1).	—	Marked reaction (2).
E.—Naked .....	(Not properly exposed).	Very marked (2).	Very marked reaction (3).

The figures in brackets represent the order of the degree of intensity of the erythema resulting from the sun exposure. It was seen that the greatest reaction was obtained from direct skin exposure, to a less extent was the result from the water-covered area ; uviol glass gave a marked reaction while that under the glass was slight ; the copper sulphate cell, which filters out the ultra-violet, gave no reaction whatever.

The experiment was repeated at the open window of a south room on the 9th December, 1922, with the sun showing a black fur temperature of 50° C., dry bulb 9, dry kata 8. After an exposure of 2 hours, no reaction was visible in any of the areas exposed. Two feet of snow had fallen and there had been severe frost, 16° C., at night ; the walls of the room (windows never shut) were cold, and it was freezing in the shade on the balcony.

*The Effect of Heat and Sunlight.*

In this experiment, the effect of exposure of the skin to the sun and also of heating the areas so exposed, by means of an electric heater, were observed. A rubber sheet with a series of circular holes 1 inch in diameter was attached to the chest. The three areas of skin exposed on the right side of the chest were exposed to the sun ; the two upper areas of the left side were exposed to the sun and also to the warmth of the electric heater ; the two lower areas of the left side were shaded from the sun and exposed to the warmth of the electric heater.

Table V.

11.12.22.

	Surface Temperature.	Time of Exposure.	Results.
Areas exposed to sun .....	35.5 C.	30 minutes.	Slight erythema.
Areas exposed to sun and heat ...	42° C.	30 "	Marked reaction.
Areas exposed to heat .....	40.5° C.	30 "	Nil after the blush due to warmth had faded away.

A similar observation was made by attaching a rubber sheet, which had two holes punched out in it, to the forearm. Two circular areas of skin, 1 inch in diameter, were exposed in this way. Over one area snow water was irrigated ; over the other, water at body temperature was irrigated. Both areas were exposed to the sun for 2 hours. The skin irrigated with the warm water showed a marked injection and erythema, the cold area failed to give any reaction.



Table VI.

12.12.22.

A.—Irrigated with snow water.

B.—Irrigated with body temperature water.

—	Time of Exposure to Sun.	—
A.	2 hours.	No reaction.
B.	2 hours.	Marked erythema.

To carry this investigation further the authors have used both the carbon arc and the vapour lamp, and carried out a number of experiments, which have shown that the time required for a lethal effect to be produced on typhoid bacilli and hay infusoria varies with—

- (1) The temperature at which the organisms are exposed.
- (2) The distance at which the organisms are exposed from the source of light.

Under the influence of the radiation, the movements become sluggish, numerous fine granules are to be seen in the protoplasm, and a characteristic vibratory movement of the cilia is noticeable before the infusoria become quite stationary and immobile, finally they completely lose their shape and become spherical and granular. Some organisms possess a greater resistance to light than others. In order to obtain a definite index for comparison, in all the experiments, the time at which the organisms demonstrated a definite change in activity—i.e., became definitely sluggish and granular with 1–2 organisms in the field immobile—was taken as the index of the effects of the light action.

In the first group of experiments, the motile organisms were cultured in tap water, and were exposed to the light in a small cell, 2 × 2 cm. and about 3 mm. in depth, made by sealing to a glass slide (by means of sealing wax) a thin piece of quartz, uvioi glass, or plain soda-glass (coverslip), or by exposing a drop of the culture in a cell direct to the light. These cells were fastened by an elastic band to the outer side of a tin can, which contained water at varying temperatures.

Table VII.

11.1.23.

(1) Typhoid B. in quartz cell (in tap water 1/10).

Temperature of tin.	Distance from M.V.L.	Time taken to become immobile.
	9 inches.	6 minutes.
CO <sub>2</sub> snow in tin.	9 ..	Active after 13 minutes.

(2) Hay Infusoria exposed to M.V.L. 7½ inches distant.

Temperature of tin.	Hanging drop exposed in air.	ed by rtz.	Covered by Uviol glass.	Ordinary microscope glass slide.
	minutes.	ites.	minutes.	
10° C.	7	8	150	+
15° C.	6	6	94	+
20° C.	5½	5	57	+
25° C.	5	4½	25	+
30° C.	3½	3	21	Active after 2 hours.

From these results it will be apparent that temperature is an important factor in this lethal action of light. However, it was impossible to obtain a definite conclusion as to the actual temperature in this small cell by these

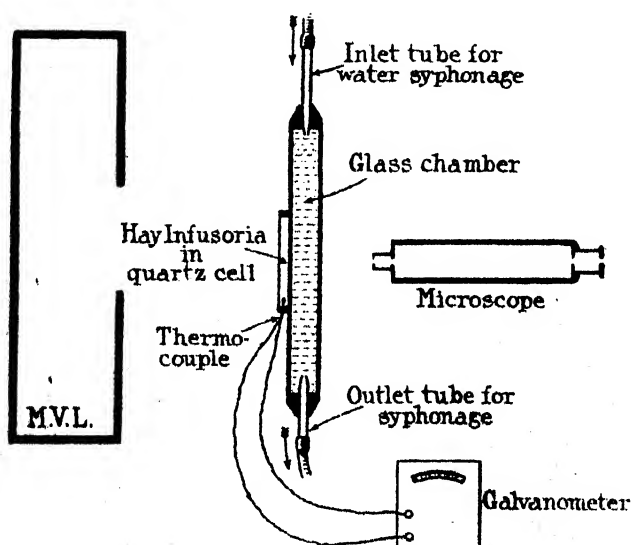


FIG. 1.

means. A special chamber was, therefore, made by fixing two glass slides together with a thick layer of sealing wax, with means to effect a continual water syphonage through this. On the front of this chamber a small cell about  $2 \times 2$  cm. and 2 mm. in depth was made by sealing a piece of quartz on to it. A small thermocouple was sealed into the chamber, and thus it was possible to regulate the actual temperature within the small cell containing the hay infusoria by means of the water syphonage, and to control any variations by observing the deflections of a galvanometer attached to the thermocouple. A microscope was placed behind the cell and the behaviour of the organisms was observed every few minutes during the whole time of the light exposure (see fig. 1).

Using this quartz cell throughout a series of experiments, the time taken to observe a definite lethal effect upon hay infusoria, with variation of the temperature and changing intensity of the source of light, was recorded.

At low temperature the organisms are sluggish, and upon long exposures the changes are so gradual that it is more difficult to define an end point, in these cases an average of the times of observing these changes was taken.

When the temperature of the quartz cell with infusoria was kept constant by means of the water syphonage, the time taken to produce a lethal result was recorded as the cell was moved nearer to or farther from the mercury vapour lamp.

In this way, it was possible to demonstrate that the intensity of light action was directly proportional to the square of the distance of the cell from the source of light, affording a biological proof, approximate it is true, of the law of inverse square, *i.e.*, if  $D$  = distance in inches,  $t$  = time in minutes,

$$\frac{D^2}{t} = K.$$

We found that the lethal time of light action is inversely proportional to the temperature of the living substance upon which it acts, when the distance is constant. If  $t$  = lethal time in minutes and  $T$  = temperature in  $^{\circ}\text{C}$ ., then

$$\text{Temperature} \times \sqrt{\text{time}} = \text{constant},$$

$$\text{or } T \times \sqrt{t} = K.$$

Table VIII.

Distance = 24 inches from M.V.L.

Water supply of syphonage = 24-25° C.

Hay Infusoria in quartz cell with thermocouple.

Time.	Galvanometer.	Temperature (equivalent).	Observations.
p.m.		° C.	
2.50	31.5	20.3	Experiment started.
2.56	29.5	19.7	Slower.
3.0	30.0	20.2	Sluggish.
3.4	31.0	20.1	Much slower.
3.9	30.0	20	Becoming immobile.
3.11	30.0	20	Granular appearance.
3.14	31.5	20.2	Many immobile and all granular.

Total time of exposure = 24 minutes.

Average temperature = 20.1° C.

Distance = 24 inches.

Distance = 24 inches from M.V.L.

Water bath of syphonage = 10-11° C.

Hay Infusoria in quartz cell with thermocouple.

Time.	Galvanometer.	Temperature (estimation).	Observations.
a.m.		° C.	
10.30	42	10.9	Experiment started.
10.45	40	11.1	Active +
10.50	40	11.1	Slower.
11.15	38	11.4	Slower.
11.30	42	10.9	Slower.
11.40	40	11.1	Much slower.
11.45	40	11.1	Sluggish, 1-2 appear granular.
11.50	41	11.0	3 in field nearly immobile.
11.55	38	11.4	Sluggish +, granular +
12.0 noon	40	11.1	Field emptying, many immobile and granular.

Total time = 85 minutes.

Average temperature = 11.1° C.

Distance = 24 inches.

Table IX.

	Temperature.	Distance from M.V.L. in inches.	Time taken to produce lethal action in minutes.	Distance <sup>2</sup> /time.
	° C.	inches.	minutes.	
T = 20-21°C.	21.0	12	6	24
	21.1	20	17	23.5
	20.1	24	24	24
	21.0	36	55	23.5
	20.8	48	95	24.25
T = 11°C.	11.7	12	23	6.3
	11.2	18	38	8.5
	11.1	24	85	6.8
	11.8	36	170	7.6
	11.3	48	235 +	
	14.7	24	62	9.3
	14.6	36	122	10.6
	14.5	12	15	9.2
	13.5	18	32	10.1
	14.0	30	95	9.5

Table X.—Exposure of Hay Infusoria to the M.V.L.

Temperature.	Distance ==			
	12 inches.	24 inches.	36 inches.	48 inches.
11° C.	23	85	170	Minutes. 235 +
15° C.	15	62	122	180 +
20.2° C.	6	24	55	95

T Temperature in ° C.	t Lethal time in minutes.	$T \times \sqrt{t}$ K.
<i>Distance = 12 inches—</i>		
11.7° C.	23	56.16
14.5	15	56.11
21.0	6	51.45
<i>Distance = 24 inches—</i>		
11.1° C.	85	102.12
14.7	62	114.6
20.1	24	98.5
<i>Distance = 36 inches—</i>		
11.5° C.	170	153.4
14.6	122	160.0
21.0	55	155.4

By combining the results of these two equations :—

$$(1) \frac{D^2}{t} = K, \quad (2) T \times \sqrt{t} = K,$$

it has been possible to show that

$$(3) \frac{T \times \sqrt{t}}{D} = K,$$

where

T = Temperature in ° C.

t = Lethal time in minutes.

D = Distance in inches.

This constant was found to be about 4.5.

D Distance in inches.	T Average temperature.	t Lethal time in minutes.	$T \times \sqrt{t}$ K
12	11.7° C.	23	4.68
12	14.5	15	4.38
12	21.0	6	4.29
18	14.5	32	4.3
20	21.1	17	4.34
24	11.1	85	4.25
24	14.7	62	4.85
24	20.1	24	4.1
36	11.8	170	4.26
36	14.6	122	4.46
36	21	55	4.31

It will be noticed that we have taken the temperature above zero Centigrade and not the absolute temperature; this is because 0° C., or according to our colleague, Dr. Brownlee, 0.5° C., is the physiological zero at which the reactions of life become arrested.

*The Effect of Temperature Variations on the Production of Erythema and Pigmentation resulting from Exposure of Skin to the Carbon Arc.*

Two circular areas, 1 inch in diameter, of the skin of the flexor surface of the forearm were exposed to the carbon arc lamp. During the whole time of exposure, one of these areas had a continuous irrigation of water at body temperature, the other an irrigation of ice water. The forearm was covered

by a large pad of rubber, secured by elastic bands, having two circular holes corresponding to the areas of skin exposed to the arc (*see fig. 2*).

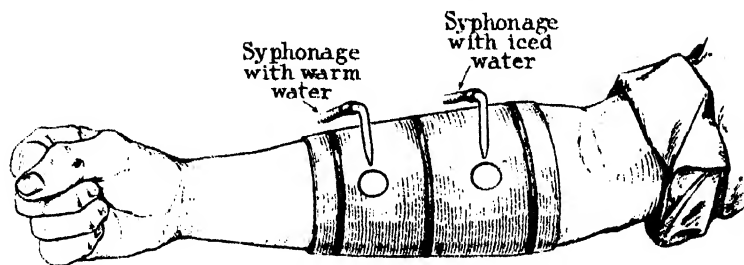


FIG. 2.

Table XI.

*Subject Miss B.*

3 Hours Exposure.	Hot Area = Irrigation of Water at Body T.	Cold Area = Irrigation with 1 c.c. Water.
15.1.23	Very marked.	"Goose" skin—injected capillaries.
16.1.23	Very intense erythema.	Slight erythema.
17.1.23	Very intense erythema.	Erythema—but less than hot area.
18.1.23	Fading.	Fading.
19.1.23	Hot area still more intense reaction than cold.	
20.1.23	Pigmentation +.	Pigmentation.

The area irrigated with hot water, showed a greater intensity of reaction and the resulting pigmentation corresponded to this phenomenon.

Three circular areas of skin were exposed by covering the forearm with a pad of asbestos, having three circular holes, each 1 inch in diameter and about 1 inch apart, corresponding to the areas of skin exposed to the carbon arc.

- (1) Area A received light from the carbon arc and also heat rays from the Garba\* placed 4 feet away.
- (2) Area B received heat rays from Garba only, being shaded from the carbon arc.
- (3) Area C received light from the carbon arc and also cold air—by passing air by means of a fan through a freezing mixture and blowing it on the skin area.

\* The Garba has a thick gas-mantle and copper reflector giving radiant heat.

Table XII.

Carbon arc, black fur = 65° C.

Length of exposure.	A.	B.	C.
12.45 12.50 Surface temperature of skin.	Injected. 41° C.	Nil. 39° C.	Experiment started. Nil. 26.5° C.
1.0	Well injected.	Slight.	Pale.
1.8	Well injected all over.	A little injection all [over.	Scattered injection.
1.15	Experiment stopped.		
2.35	Restarted.		
2.10	Erythema marked.		
5.30	Blister and general injection.	Very slight patching injected.	Almost nil.

The following day the area A showed marked reaction and blistering, the areas B and C quite disappeared and showed no reaction. The area A pigmented later on.

	T.	Time of exposure.	
A = Carbon arc and heat from Garba	41	minutes. 260	Marked injected and pigmentation.
B = Heat from Garba	39	260	Slight and finally nil.
C = Carbon arc and cold air	26.5	260	Nil.

A similar experiment was carried out by attaching a rubber sheet having two holes punched out, to the skin of the forearm. The two areas of skin so exposed were placed in front of the carbon arc lamp. The black fur showed at temperature of 62° C. over both areas. The area A (fig. 3, on Plate 11) had a current of hot air passing over it, this was effected by putting a coil of metal tubing into a large case of hot water, and passing a current of air from an electric motor inlet fan through it. The area B had a current of cold air passing over it, by a similar means, but placing the coil of metal tubing into a freezing mixture. The hot area showed a marked erythema and intense injection on the following day. The cold area only showed a slight reaction, which later disappeared. The hot area showed pigmentation 11 days after the light exposure.

15.2.23. P.

	Black Fur T.	Time of Exposure.	
	° C.	minutes.	
A.	63	105	Erythema and pigmentation.
B.	62	105	Erythema and slight.



Another experiment on the same lines was made as follows. One area of skin was exposed for 10 minutes to the M.V.L. at a distance of  $10\frac{1}{2}$  inches in the laboratory temperature,  $16^{\circ}$  C., a second area in the cold room at a temperature of  $-2.0^{\circ}$  C., a third area in the hot room at  $37^{\circ}$  C.

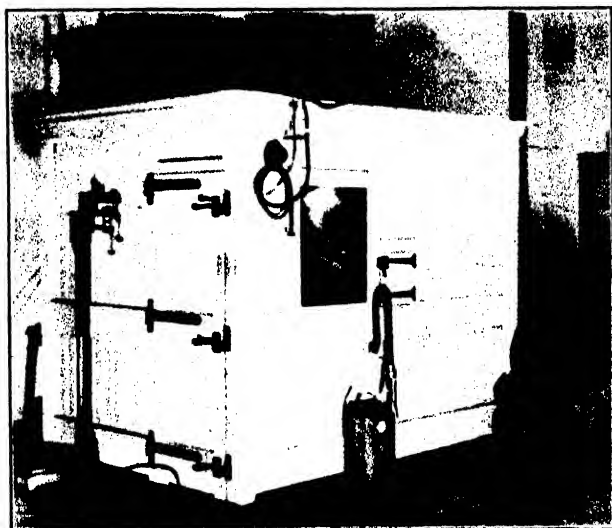
The resulting erythema was much the most in the case of the hot room, the cold room exposure gave very slight effect. Pigmentation and peeling of the skin followed only in the area of hot room exposure.

After the completion of this paper we have received one from which we take the following\* :—For paramoecia at  $14^{\circ}$  C., the lethal dose of 13.4 mg. radium (held enclosed in a thin-walled glass capsule 2 mm. away from water containing the cells) was 10 hours; at  $37^{\circ}$  C.  $1\frac{1}{2}$  hours. For each rise of  $8^{\circ}$  C. the length of the lethal dose is halved. At lower temperatures the lethal dose is somewhat shorter than would be expected. The curve is the same as that showing effect of temperature on rapidity of cell division and of pulsating of the contractile vacuole. The influence of temperature is thus of the same kind for ultra-violet and radium.

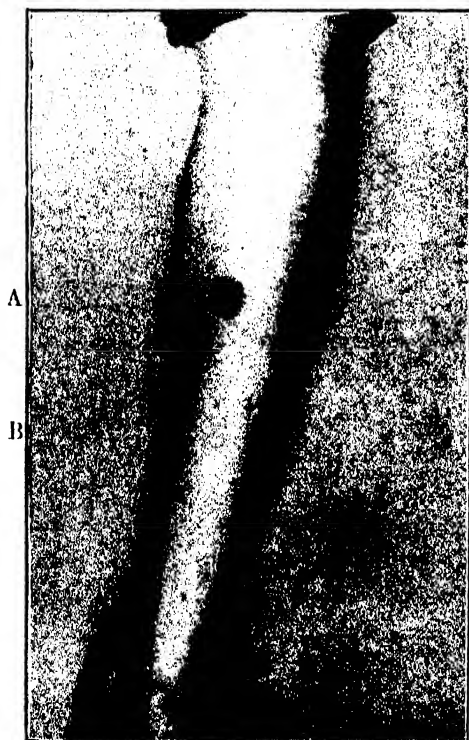
We find that the temperature coefficient for infusoria is nearly 3 between  $10^{\circ}$  C. and  $20^{\circ}$  C. Since the temperature coefficient of a pure photo-chemical reaction is very low, about 1°, the results suggest that temperature is accelerating a secondary effect of the nature of a chemical reaction. The temperature coefficient for the velocity of ordinary chemical reaction is 2.0–3.0. The amount of substance formed by light may be a little less at the lower temperature—photo-electric action is independent of temperature *in vacuo*—but the rate of reaction between it and some constituent of the living tissue may be affected in the degree usually associated with the action of heat on chemical reactions. The higher temperature may be less favourable than the lower to the stability of the infusorium protoplasm.

Turning to the consideration of excessive cold, we find that this also lessens the resistance of infusoria to the ultra-violet rays. Thus, on circulating a freezing mixture at  $2^{\circ}$  C. through the glass chamber, the infusoria in the attached quartz chamber were killed in 4 minutes by an exposure to the mercury vapour lamp, which killed them in 10–12 minutes at room temperature. The cold slowed down the movements of the infusoria greatly, but did not, by itself, kill them, not at least during a 30 minutes' observation.

\* C. Packard, 'Soc. Exp. Biol. Med.', vol. 20, p. 226 (1923).



Moss—Fig. 1.



Hill and Eidenow—Fig. 3.



Moss—Fig. 2.



*Some Effects of High Air Temperatures and Muscular Exertion upon Colliers.*

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(PLATE II.)

This paper deals with the work undertaken by the author while he was Tyndall Research Student of the Royal Society. The investigations fall under the following four distinct headings:—

1. The Food Consumption of Colliers and its relation to Underground temperature conditions.
2. Respiratory Exchange of a Collier during work.
3. Relation of High Temperatures and Work to Sweating.
4. Miners' Cramp.

Each branch of investigation is related to underground temperature conditions, and has in consequence a bearing upon each of the other sections.

*Part I.—Food Consumption.*

In order to obtain data as to food consumption a form [of which a copy with instructions attached accompanied the manuscript of this paper] was sent, together with a "trade" spring-balance weighing by  $\frac{1}{4}$  quarter-ounces to four pounds, and a Fahrenheit thermometer, to seventy colliers working at the coal face in different parts of the country. Sixty forms were filled up in such a manner as to justify their inclusion in the records of this investigation. A surprising amount of trouble and care was taken in the filling up of these forms. The men selected had in every case a reputation for steadiness and good work, and wherever possible were also attending evening classes. The actual duration of the work at the face would be about

The total number of forms will appear few in comparison with the colliers in the respective districts, but it is to be doubted whether any material difference would have resulted by obtaining many more. It is extremely difficult to induce even the best men to undertake the required task for seven to ten days. In fact, many more refused than accepted; and

often, when it was possible to find a man willing to fill up the form, his intelligence was not such that he could be trusted to obtain and record accurate information.

The calorie values of most foods, cooked and uncooked, were taken direct from the figures given by Graham Lusk in his book 'The Science of Nutrition' (1920), and a few were taken from 'Analysis and Energy Values of Foods' by Dr. R. H. A. Plimmer. It was necessary, however, to calculate the calorie value of a number of standard articles of dietary after first ascertaining the loss or gain in weight due to cooking. In this work the author was assisted by his wife.

Before giving a tabulated statement of the results obtained, it will be of interest to quote the following calorie values of food eaten by various manual workers, as estimated by Atwater from the general results of his well-known investigations :—

	Cals./day.
Men at moderate work consumed .....	3500
„ hard „ .....	4500
„ severe „ .....	5700

The following instances show, however, that sometimes the food consumption was still greater :—

	Cals./day.
Blacksmiths at hard work actually consumed .....	6905
Teamsters, etc., at severe work .....	7805
Brickmakers „ .....	8805

Table I shows the average results obtained and the relationship which was found between the colliers' consumption of food and drink in relation to temperature. The forms were collected over a period of 24 months—March, 1920–1922—during which time changes in the wage rates took place. It is unlikely, however, that this could affect the results, because during periods of low wages the ration of the breadwinner must be maintained to enable him to carry out his daily work in the mine.

It appears from the figures shown that the workers in hot mines consumed more food than those working in cool places. The average daily calorie consumptions of the colliers working at the warmest colliery are shown in Table I to be 5925; but two of the forms returned gave very abnormal calorie values. Had they been excluded, the average for the colliery would have worked out at 4942 calories per man per day. Column 9 gives the calorie

Table I.

District.	Percentage Calorie Value of Principal Foods to Total Calorie Value of Food eaten per day.																		
	1	2	3	4	5	6	7	8	9	10	11	12							
	No. of Forms Returned.	Average Period covered by each form in days.	Average Underground Temperature in ° F.	Month of Year in which forms were filled up.	Average Height of the Men in cm.	Average Weight of the Men in kg.	Average Body Surface Area in square metres.	Average Calorie Value of Food eaten per man-day.	(Calories per square metre body surface.	Wages Standard on the basis of the highest.	Average Total Liquid drunk per day in pints.	Bread.	Butter, Mar- garine, Drip- ping.	But- cher's Meat.	Ham and Bacon.	Pota- toes.	Pud- ding.	Cake.	Cheese.
Pendleton ..	6	10	99	May	172	62.7	1.75	5925	3386	82	11.9	32.6	15.5	13.8	9.6	5.5	1.4	0.8	3.1
Pendlebury ..	6	10	88	July	163	60.4	1.68	5114	3044	82	9.2	32.1	11.4	14.1	16.3	6.6	1.1	2.2	2.9
Hanshead ..	10	9.1	79	June- Sept.	170	70.0	1.81	4644	2566	80	8.5	31.9	7.8	15.8	18.6	7.2	1.0	1.3	5.7
Forest of Dean	12	7.2	68	Dec.- Jan.	169	63.1	1.72	4293	2494	55	3.6	33.4	11.1	11.9	6.6	9.3	5.6	4.5	4.3
Derbyshire ..	8	10	66	March	169	59.9	1.69	4211	2491	100	4.4	34.2	7.8	10.9	9.9	5.5	9.9	3.9	1.5
Yorkshire ..	8	7.6	59	Feb.- July.	169	65.0	1.75	4472	2555	90	4.6	37.5	15.0	7.3	4.0	5.4	8.7	3.5	0.4
South Wales	10	7.9	55	Dec.- Jan.	170	65.3	1.76	4320	2454	88	3.7	31.2	17.4	11.0	11.2	3.5	2.1	12.1	2.0
Mean figures for the whole												33.3	12.3	12.1	10.9	6.1	4.2	4.0	2.8

consumption for the respective districts worked out on the basis of calories per square metre body surface, as determined by Du Bois's formula. It will be noticed that the relative figures are not much altered on this basis of comparison. The percentage proportions on a calorie basis of the main foods eaten are interesting. The figures given in Columns 8, 9, 11 and 12 are the daily averages for the whole period covered by the forms and are, therefore, somewhat lower than the averages which would obtain had all the days covered by each form been work days. A collier at Pendleton, for example, drank 13.6 pints (17.0 lbs.) of liquid per working day, as against 11.9 pints (14.9 lbs.) shown in Table I.

Table II gives figures showing the amount of sodium chloride eaten by the colliers in the various districts, apart from what they deliberately added to their food. Although this table does not pretend to give the total salt consumed it gives the approximate total salt content of the principal foods constituting the dietaries. This has a significant bearing upon the work to be reported on later in connection with the regulation of body temperature. It will be seen that the workers in the hot mines consumed much more of salted food (ham and bacon).

Table II.—Sodium Chloride consumption per day.

Food.	Per cent. NaCl Content Hamill, Pillmer, and K.N.M.	DISTRICTS.													
		Pendle- ton.		Pendle- bury.		Ham- stead.		Forest of Dean.		Derby- shire.		Yorks.		S. Wales.	
		Wt. in gms.	NaCl in gms.	Wt. in gms.	NaCl in gms.	Wt. in gms.	NaCl in gms.	Wt. in gms.	NaCl in gms.	Wt. in gms.	NaCl in gms.	Wt. in gms.	NaCl in gms.	Wt. in gms.	NaCl in gms.
Bread .....	1.0	762	7.6	624	6.2	567	5.7	550	5.5	558	5.6	582	6.3	510	5.1
Butter and margarine...	1.25	130	1.6	77	0.9	51	0.6	62	0.8	41	0.5	85	1.0	99	1.2
Bacon and ham .....	5.0	85	4.2	116	5.8	119	5.9	37	1.8	57	2.8	28	1.4	77	3.8
Meat .....	0.1	221	0.2	241	0.2	224	0.2	164	0.2	178	0.2	107	0.1	186	0.1
Cheese .....	2.0	40	0.8	84	0.7	62	1.2	42	0.8	14	0.3	8	0.1	20	0.4
Cake and biscuits ..	0.6	17	0.1	40	0.2	23	0.1	62	0.4	57	0.4	51	0.3	178	1.0
Potatoes, cooked .....	0.6	317	1.9	380	2.3	340	2.2	420	2.5	224	1.3	244	1.5	162	0.9
Vegetables ..	0.6	79	0.5	82	0.5	57	0.4	99	0.6	40	0.2	31	0.2	45	0.3
Total salt in gms. ....			16.9		16.8		16.8		12.6		11.8		10.9		12.8

The main conclusions may be summed up as follows :—

1. The mean daily calorie consumption of the colliers was 4711. This is in accordance with what might be expected from the observations of Atwater and others on the food consumption of men engaged in hard muscular work.

2. The food consumption increased with increase of temperature underground. This is not the result which was expected, since a high air temperature and particularly a high wet-bulb temperature tends to diminish a man's capacity for hard work. The increased physiological work involved in the extra sweating at the higher temperature may possibly account for the extra metabolism, though this seems hardly probable.
3. The workers in the hot mines consumed a larger amount of salted foods than those working under lower temperature conditions. As will be shown later this is probably related to the loss of salt in sweating. The proportion of butcher's meat eaten was also greater in the hot mines.
4. The amount of liquid drunk increased rapidly with increase of the temperature above 70°. This is, of course, related to the amount of sweating.

*Part 2.—Respiratory Exchange of a Collier during work.*

The collier upon whom the following experiments were conducted is a man 5 feet 7 inches high, 148 lbs. in weight (stripped), with a food consumption which was found to be 6028 calories per day. His basal metabolism was calculated from Du Bois's formula to be 1.2 calories per minute. The quantity of air breathed during a whole shift was first of all determined by letting him inspire air through a meter. The method employed was as follows:—

To the man's mouth was attached a rubber mouthpiece fitted with face straps and nose clip as used in mine rescue apparatus. To the mouthpiece was attached a Rosling valve, and on the inspiratory and expiratory sides of it were attached 3-foot lengths of flexible rubber tubing. On the inspiratory side an air meter was connected by means of which the quantity of air breathed by the man could be read to within a ten-thousandth of a cubic foot. When a sample of expired air was required a Douglas bag of 100 litres capacity was attached to the end of the expiratory tube. When the bag was nearly full it was disconnected, and after well mixing the contents by pressing the sides of the bag in and out, the air was expelled through a gas sampling tube, so that a representative sample was secured.

The time and reading of the meter when changes in work took place (as, for example, the change from loading to coal hewing) was noted, so that the air consumption per minute during any given piece of work was determinable.

Before the actual experiments were begun the subject was thoroughly accustomed to the use of the mouthpiece. The experiments were continuous



over the whole of three working shifts, except for a short break each day which was allowed for a meal. The barometric and temperature conditions were noted, and in all cases the volumes recorded are reduced to N.T.P. The quantities of air breathed as shown below are the average results for the three experiments.

	Litre/min.
Quantity of air breathed during the whole shift, including periods of rest.....	28.4
Quantity breathed during actual work .....	30.975
"        "        periods of rest.....	16.37
"        "        cutting with pick in solid coal....	35.333
"        "        loading slack with basket and rake	36.2
"        "        getting coal with pick.....	29.858
"        "        timbering (hacking and setting)..	28.04

On the third day samples of inspiratory and expiratory air were taken during specific pieces of work, the results of which are summarised in Table III as follows :—

Table III.—Analysis and Volumes of Inspired and Expired Air.

Nature of Work.	Bar.	Dry Bulb ° F.	Wet Bulb ° F.	Volume at N.T.P. dry air.	Inspired Air.		Expired Air.		O <sub>2</sub> consumed. Per cent.	CO <sub>2</sub> expired. Per cent.	CO <sub>2</sub> in litre/min. at N.T.P.	O <sub>2</sub> in litre/min. N.T.P. dry air.	R.Q.	Calc. expended per min. 1 litre O <sub>2</sub> = 4.74 cal.
					O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>						
Cutting in bottoms for props .....	32.14	80.6	78.8	29.82	19.98	0.19	15.99	4.21	3.98	4.02	1.178	1.168	1.01	5.536
Loading slack .....	32.14	81.1	78.9	40.35	19.86	0.38	15.04	4.83	4.91	4.46	1.795	1.96	0.91	9.835
Cutting timber with axe and erecting .....	32.14	81.3	79.0	31.346	19.86	0.38	15.58	4.75	4.28	4.37	1.370	1.334	1.02	6.323
Loading slack .....	32.14	81.85	79.2	38.679	19.86	0.38	15.06	4.51	4.96	4.12	1.592	1.917	0.83	9.082

After the above data had been obtained under actual working conditions the same collier attended the University on three evenings, when experiments were performed, a summary of which is shown in Table IV.

Table IV.

No. of Experiment.	Nature of Occupation.	Temp. ° F. dry.	Temp. ° F. wet.	Air breathed in litre/min.	Work done in ft./lbs. min.	CO <sub>2</sub> expired in litre/min.	O <sub>2</sub> consumed in litre/min.	R.Q.
1	Rest in an arm-chair .....	63.5	59.0	11.46	—	0.323	0.362	0.893
2	Rest in an arm-chair .....	90.9	78.3	9.74	—	0.30	0.273	1.10
3	Working on a Martin's ergometer....	98.7	78.3	15.06	960	0.665	0.735	0.905
4	Working on a Martin's ergometer....	97.0	86.3	25.3	2596	1.088	1.138	0.956
5	Working on a Martin's ergometer....	95.4	85.0	32.0	3576	1.296	1.38	0.94

It will be seen from experiments 3, 4 and 5 (Table IV) that the increase of oxygen consumption is 0.0246 c.cs. for every additional 100 ft./lbs. of work done per minute on the ergometer, above the basis figure 735 c.cs. oxygen for the expenditure of 960 ft./lbs. min. The ergometer equivalents for work done whilst carrying out specific pieces of work in the mine can, therefore, be deduced from the figures for oxygen consumption given in Table III, and are as follows:—

1. Cutting in bottoms (hewing coal in the floor)—

$$(1.168 - 0.735) + 0.0246 = 1760 + 960 = 2720 \text{ ft. lb./min.}$$

2. Loading slack—

$$\left. \begin{aligned} (1.98 - 0.735) + 0.0246 &= 5061 + 960 \\ (1.917 - 0.735) + 0.0246 &= 4806 + 960 \end{aligned} \right\} = 5893 \quad ,,$$

3. Cutting and erecting timber—

$$(1.334 - 0.735) + 0.0246 = 2435 + 960 = 3395 \quad ,,$$

The collier upon whom these experiments were conducted has the reputation for being the best worker in the colliery (a South Staffordshire one), his average for tub-filling being double the average for all underground fillers in the same pit. It is likely, therefore, that average figures for colliers as a class would more nearly conform with the following figures, which are mainly empirical, being based upon the author's experience of the work of this man in comparison with the average coal-getter—

1. Cutting in bottoms..... 2500 ft. lb./min.
2. Loading slack ..... 4000 ,,
3. Cutting and erecting timber ..... 3000 ,,

These figures are of interest in that they are the first to be determined, and will form a useful basis for comparison with work done in other industries. It must be understood, however, that they are merely the ergometer equivalents of the work done by one particular man.

*Part 3.—The Relation of High Temperatures and Work to Sweating.*

The preliminary part of this work was carried out in Prof. J. S. Haldane's laboratory at Oxford in June, 1920, and in August of the same year experiments were conducted in the experimental chamber at the Doncaster Coal Owners' Mine Rescue Station. The Oxford experiments can be summarised briefly as follows :—The writer sat in as restful a position as possible in the respiration chamber, which was heated electrically. The body was protected against radiant heat from the heaters, and apart from the convection currents set up, there was no circulation of air in the chamber.

The following Table I gives the results of a series of experiments conducted at rest, fasting, there being no passage of urine or fæces, and any increase in weight of clothing being counted as body loss.

Table I.

Chamber Temperatures.		Body loss in lbs. per hour.	Remarks.
Dry Bulb Temp. ° F.	Wet Bulb Temp. ° F.		
68	—	0·095	During this experiment the mouth temperature rose 2·2° F., indicating that sweating was being stimulated to a maximum extent.
81	—	0·139	
82	—	0·152	
98·6	75	0·240	
110	84	0·430	
118·4	85·6	0·85	
123	89·3	1·115	

A series of experiments was carried out to indicate the relation of sweat loss to work done. The work was performed on a Martin's bicycle ergometer placed in the laboratory, the air temperature of which varied between 60° and 67° F. Table II gives the results obtained :—

Table II.

Ft./lbs. of work per minute.	Loss in lbs. per hour.
709	0.18
2443	0.53
3420	0.736
5130	1.45

The loss shown in Tables I and II represents the gross loss by weight, almost the whole of which is due to the evaporation from the skin and lungs and moisture caught by the clothing. The weight of oxygen taken in compensates almost completely for the weight of carbon dioxide exhaled.

The Doncaster experiments conducted by Mr. J. Ivon Graham and the writer demonstrated the inability of unacclimatised persons to sweat to anything like the extent to which, as will be shown later, a collier acclimatised to high temperature can sweat. It was, in fact, found impossible to produce by sweating a loss of as much as  $1\frac{1}{2}$  lbs. per hour, as shown in the following table :—

Table III.

Subject.	Experiment No.	Chamber Temperature.		Body Temperature.	Air Velocity in ft. per min.	Loss in lbs./hour.
		Dry Bulb ° F.	Wet Bulb ° F.			
J. I. G.	1	106.5	82.8	97.9—99.9	—	0.594
	2	105.0	82.0	98.2—98.4	185	1.125
	3	107.6	85.0	98.4—99.2	183	1.437
	4	108	89.2	99.8—101.6	790	1.2
	5	110.5	81.0	99.0—100.1	800	1.437

Experiments Nos. 4 and 5 were interesting in that the hot air current of approximately 800 feet per minute dried up the skin, so that J. I. G., unable to evaporate moisture fast enough, felt very uncomfortable, and experienced a sharp rise in body temperature.

Owing to the move of the Doncaster Coal Owners' Research Laboratory to the Mining Department of Birmingham University, and the time taken in erecting a new respiration chamber, the experiments were postponed until July, 1922. In the meantime, however, information was obtained showing the actual loss in weight by sweating of colliers working in hot mines. This

was determined by weighing the men dressed, plus their full water bottles and snap, before they entered the pit. They were reweighed after the shift's work, and the difference in weight, less the amount of urine passed plus the amount of moisture absorbed by the boots and clothing (if worn), gave the amount of moisture lost by perspiration and respiration. The moisture in the working clothes and boots was determined by weighing them dry and wet. The results for Hamstead Colliery are as follows :—

Table IV.—Hamstead Colliery Results.

Underground Temperature.	Subjects.	No. of separate observations.	Max. loss of weight in lbs.	Min. loss of weight in lbs.	Average loss of weight in lbs.	Loss of weight in lbs./hr. on the basis of a 5½-hr. working period.
82° F. dry 77° F. wet	D. T. ....	28	11.81	8.25	9.35	1.7
	A. C. ....	1	—	—	10.19	1.85
	R. S. ....	5	9.25	7.12	7.81	1.42
	W. T. ....	4	7.25	5.19	6.44	1.17
	C. P. ....	3	9.06	5.94	7.31	1.33
	W. D. G. ....	1	—	—	6.62	1.20
	K. N. M. ....	1	—	—	4.69	0.85

The man, D. T., had the reputation for being the best worker in the pit. The cases K. N. M., with a student assistant W. D. G., are interesting in that both were unaccustomed to manual work; and in order to compare the effects of work with that of a collier, each loaded five tubs of coal and four tubs of slack, which is a fair day's work. During the first two hours of the work period, each perspired visibly and freely, but afterwards the body appeared quite dry, although the body temperatures remained normal in each case.

Another interesting observation was made in connection with the experiments upon D. T., who worked for one shift in a thick cloud of coal dust. The dust collected on his body and formed a thin covering which throughout the shift appeared fairly dry. On this occasion he lost nearly 1 lb. less in weight than on the previous day, although he worked equally hard, if not harder. He stated that when his body was covered with coal dust he did not perspire so freely. It seems probable that the layer of coal dust on his skin prevented sweat from dripping off him and so being wasted as regards its cooling influence. Another interesting observation by the same man was to the effect that when he recommenced work after several weeks' holiday he was unable to maintain his normal rate of sweating, with the result that during

the last two hours of the shift he was good for nothing. His temperature had presumably risen. This condition only lasts two days at the most.

The results for Pendleton Colliery are shown in the following table :—

Table V.—Pendleton Colliery.

Underground Temperature ° F.	Case No.	Loss from skin and lungs in lbs.		Hours worked.		Average loss by evaporation in lbs. per hour for the two shifts.
		1st day.	2nd day.	1st day.	2nd day.	
98° to 100° F. dry bulb, and 85° F. wet bulb.	1	18.56	15.25	5	5.75	3.175
	2	10.44	12.94	5	5.75	2.168
	3	18.75	18.0	5.5	6.25	3.145
	4	13.25	11.62	5.5	6.25	2.135
	5	15.44	16.12	5	6.25	2.695
	6	12.12	12.44	5	5.75	2.28
	7	12.68	10.81	5	5.75	2.205
	8	9.68	13.75	5	5.75	2.16
	9	13.68	12.75	5	5.75	2.475
	10	9.31	9.37	5	5.75	1.76
	11	11.31	12.12	5	5.75	2.185
	12	10.56	10.12	5	5.75	1.93
	13	11.81	17.12	5	5.75	2.67

It will be seen that of the 13 men the maximum individual loss from the skin and lungs amounted to 18.56 lbs. for five hours' work, and the minimum loss amounted to 9.375 lbs. for 5.75 hours' work ; or, in other words, the loss in lbs. per hour was 3.7 and 1.66 respectively.

The average loss per hour by skin and lungs for the 13 men during the two days will be seen to be 2.38 lbs., and the average work period 5½ hours. The average volume of urine passed per man was 155 c.c. This is only half the average passed during ordinary conditions at normal temperature, and the water drunk during the shift amounted on the average to 7½ lbs. so that it did not compensate for the loss by skin and lungs, nearly all of which must, of course, have been from the skin.

As the sweat contains a very appreciable amount of chloride it is evident that the miners were losing much chloride during this work. It is obvious that this subject is of sufficient importance to merit thorough scientific investigation.

Further investigations on sweating have been commenced in the respiration chamber erected in the Mine Rescue Room, Birmingham University, in connection with the Mining Research Laboratory. This chamber of 268½

cubic feet capacity can be heated electrically by radiators to a temperature above 130° F. (Plate 11).

The photographs show (fig. 1) outside view of chamber and (fig. 2) ergometer. The fan, which can be regulated to run at various speeds, circulates a current of air in the chamber which can be varied from 0 to 17 cubic feet per minute. The water bottle A is filled with warm distilled water and connected by rubber piping to a hose which is put through the air exit to enable a man to wash himself down inside the chamber. By this means a man can cease work, wash down, dry and recommence work in just over 10 minutes. A Martin bicycle ergometer is used for accurate work measurement. Underneath the ergometer is placed a mackintosh sheet to catch all sweat dropping from the man whilst peddling. After each experiment the ergometer and stand is thoroughly washed down with distilled water, which collects in a large galvanised iron tray. The man himself is also washed down into the tray, the total washings measured, and the chlorides estimated as sodium chloride, determined by Volhard's method. It has often been assumed that the chloride in sweat is almost entirely sodium chloride, since sodium predominates very greatly over potassium and other basic substances in the blood plasma. An analysis made by Mr. J. Ivon Graham in connection with one of these experiments showed, however, that 59.3 per cent. of the chloride was sodium chloride and the remaining 40.7 per cent. potassium chloride.

Before commencing an experiment the subject is washed down very thoroughly with hot water, dried and weighed after first passing urine. The work is performed in thin cotton shorts, washed in distilled water, dried and weighed before use. Exact weights are taken of food eaten, water drunk and urine passed during any given experiment. The volume of air expired is measured by an air meter during the performance of a given amount of work and also during rest. This information enables one to estimate approximately the moisture loss by respiration and obtain the loss due to perspiration only. The loss of perspiration in lbs. per hour, as shown in the table, is, therefore, actual; the respiration loss having been deducted. The latter loss was, however, very small.

The experiments were carried out upon the three following subjects:—

A. P. V., who is a member of the teaching staff of the Mining Department. He cycles 14 miles each day and, therefore, can be considered fit, but not accustomed to manual work. F. R., a mechanic assistant in the Mining Department, a man of good physique and standard of intelligence much above the average. S. C., a young collier, who for the past 4½ years has worked in

Pendleton pit, the last year having been spent filling tubs at the coal face in the hottest district. This man is fully acclimatised to heat, and forms an interesting contrast with F. R. and A. P. V.

From these experiments the following conclusions result (see Table pp. 194 and 195).

1. The sodium chloride content of the sweat as determined is much lower than the figure generally given in text-books. Thus Luciani, who in his 'Physiology' gives a full account of existing knowledge in relation to sweating, gives the percentage of sodium chloride in sweat as about 0.6. The maximum and minimum percentage contents as determined in these experiments will be seen to be 0.325 and 0.118 respectively, with an average of 0.224 per cent. This result confirms the findings of Dr. E. H. Hunt in experiments conducted by him at Oxford. Dr. Hunt collected sweat with careful precautions to prevent errors from evaporation and previous contamination of the skin. (See 'Journal of Hygiene,' vol. 12, p. 479 (1913).)

2. For fixed conditions of temperature and humidity an increase in the work output is accompanied by an increase in the sweat loss, and apparently also of its sodium chloride concentration. Also, if the work output be maintained constant, an increase in the loss of sweat and its sodium chloride content accompanies an increase in temperature. Where there is much sweating the loss of chloride from the body is very large and may be enormously greater than the loss by the urine. This great loss of chloride by sweating is evidently related to the extra quantity of salt in the diet of miners working in hot mines. It also throws clear light on the requirements for salt and the incidence of a salt tax in a hot climate.

3. There is a marked difference in the amount of sweating between the man acclimatised to hard manual work under high temperature conditions, and men who take just sufficient exercise to keep fit. It will be seen that for the same work output under nearly equal conditions of temperature and humidity the collier loses more than twice as much weight by sweating as does A. P. V. When, in a further experiment, the collier was pressed by an increase in the dry and wet bulb temperature he lost 5.8 lbs. per hour by sweating, which is a remarkable figure. I am enabled to quote evidence from Dr. Hunt in the same direction. In a letter from Singareni, India, to Dr. J. S. Haldane he gives the following information: (1) While taking violent exercise, such as a hard single at tennis, in an air temperature of, say, 102° F., or a little more, the loss of body weight is 4 lbs. per hour, as against the loss of 2 lbs. in the Oxford Turkish Bath. (2) A healthy 110-lb. weight Dravidian coolie can



*Summary*

Observed Data.	Water drunk during Experiments.								
	A. P. V., University Lecturer.			F. R., Mechanic.		S. C., Collier.			
Ft. lbs. of work done per minute ....	2682	3353	3687	3353	4695	2816	3353	4024	5365
Dry bulb temperature ° F. ....	96	95.2	95.6	113.8	112	111.4	104.2	103	102.8
Wet bulb temperature ° F. ....	70.1	70.5	70.2	79.5	80.7	81.8	82.8	85.2	86
Average loss of sweat in lbs. per hour	1.14	1.18	1.09	1.43	1.62	2.24	2.42	3.28	3.84
Average weight in lbs. of water drunk during experiment.	3.16	3.69	4.83	4.64	1.125	5.56	6.31	8.69	7.81
Average period worked in hours.....	3	3	3	3.33	2	3	3	3	2
Average weight in grains of salt taken	—	—	—	—	—	—	—	—	—
Average volume of urine passed in cubic cm.	146	919	1039	330	83	74	94	55	77
Average percentage of chloride (NaCl) content of sweat.	0.223	0.289	0.165	0.207	0.325	0.189	0.187	0.259	0.199
Average percentage of chloride (NaCl) content of urine.	0.778	0.204	0.258	0.613	0.88	0.676	0.546	0.389	0.282
Average total salt content of sweat..	3.473	4.668	2.448	3.913	4.364	5.867	6.410	11.575	6.936
Average total salt content of urine..	1.136	1.804	1.715	1.232	0.731	0.482	0.577	0.257	0.327
Average total salt loss during experiment.	4.609	6.472	4.163	5.145	5.095	6.349	6.987	11.832	7.263
Ratio between salt excretion of sweat glands and that of kidneys.	3.06	2.6	1.43	3.17	5.97	12.2	11.1	45.0	21.2

lose 10 lbs. weight in five hours without any apparent symptoms, though this represents 10 per cent. of his total body weight (109 lbs. to 99 lbs.). He could doubtless lose more before he commenced to suffer badly.

4. The distribution of chloride excretion between the kidneys and the sweat glands is of considerable interest. It will be seen that in the cases of F. R. and more particularly S. C. there was a very marked relative increase in the excretion of chloride by the sweat glands with increase of work at high temperatures, whereas in the case of A. P. V. there was no such increase.

The subject of this part of the paper is receiving further attention in order to clear up points not sufficiently established. For this reason a fuller statement of a number of the data obtained is withheld for the present.

*Part 4.—Miners' Cramp.*

*Miners' Cramp* has hitherto been observed only among the workers in hot mines where the temperature varies between 98 and 102° F. dry bulb and 83° to 87° F. wet bulb. In fact, the writer does not know of pits other than Agecroft and Pendleton where cases have been reported. There seems to be no doubt, however, that even where cases of severe cramp do not occur, miners may be partially disabled in respect of working capacity by the same

*of Results.*

No Water drunk during Experiments.										Salt Water drunk.				
A. P. V., University Lecturer.						F. B., Mechanic.		S. C., Collier.	A. P. V., University Lecturer.			F. B., Mech.	S. C., Collier	
2682	2682	3353	3353	3687	3687	2213	3353	4095	3353	2682	3353	3687	—	5365
71.6	94.7	71.0	95.3	69.5	96	108	115.5	114	103	94.9	96.3	95.3	—	101
59.2	69.3	57.0	69.8	57.1	69.7	79.7	79.7	81	85	70.0	70.3	70.0	—	85
0.31	1.06	0.42	1.10	0.46	1.09	1.02	1.29	1.718	2.45	1.07	1.16	1.05	—	3.08
—	—	—	—	—	—	—	—	—	—	3.43	3.68	3.62	—	6.56
3	3	3	3	3	3	3	3	2	3	3	3	3	—	2
—	—	—	—	—	—	—	—	—	—	4.61	6.47	6.0	—	0.32
165	110	212	122	195	170	150	65	60	100	350	130	126	—	75
0.118	0.286	0.144	0.256	0.196	0.24	0.200	0.28	0.305	0.227	0.224	0.223	0.182	—	0.246
1.33	1.614	1.556	1.536	1.591	1.158	1.121	1.287	0.50	1.392	0.585	1.62	1.584	—	0.234
0.502	3.473	0.828	3.843	1.238	3.577	2.573	4.568	4.36	7.58	3.266	3.533	2.596	—	6.871
2.2	1.136	3.3	1.896	3.102	1.968	1.666	0.836	0.30	1.392	2.047	1.976	2.005	—	0.175
2.702	4.609	4.128	5.739	4.34	5.545	4.239	5.404	4.66	8.972	5.313	5.509	4.601	—	7.046
0.228	3.06	0.25	2.03	0.40	1.82	1.54	5.46	14.5	5.45	1.6	1.8	1.3	—	39.3

cause as leads finally to attacks of cramp. This subject is of general importance, therefore, in connection with deep mining problems of the future, and in connection with other industries where men are performing hard work at high temperatures.

The number of cases severe enough to warrant the carrying of the men out of the pit was only nine in two years, but minor attacks of cramp occurred more frequently, and would no doubt have developed into severe cases had not the men ceased work immediately.

The evidence taken from sufferers and non-sufferers working in the same district leads one to attribute cramp to the following causes :—

- (a) High air temperatures.
- (b) Excessive drinking of water due to (a).
- (c) Continued hard work.

Men are generally affected by cramp during the second half of the shift and always in the muscles actually being strained at the time. Sufferers are generally men of poor physique. If a man is attacked whilst lifting a full tub on to the rails, cramp might occur in the arms, legs or abdomen ; if the latter, the man is put out of action immediately, the contortion of the abdominal muscles being so great as to form a lump the size of a cricket ball. In severe

attacks of cramp it may take half a dozen men to hold down a sufferer and straighten out the affected limbs. Such treatment produces excessive exhaustion of the sufferer, and sal volatile is usually administered to revive him. The evidence collected from the men in the pit was submitted to Prof. J. S. Haldane, who suggested that cramp may depend upon excessive loss of chloride by continued sweating. The matter was then investigated by Mr. J. B. S. Haldane, who accompanied the writer and Prof. A. V. Hill, F.R.S., to a deep coal face at Pendleton Colliery and examined the urine. A sample of urine obtained at the end of the shift from one of the colliers who was subject to cramp was found to be practically free from chloride, though very little urine was being passed. It gave not even the slightest cloudiness with silver nitrate, though only 5 c.c. were secreted during  $4\frac{1}{2}$  hours. This phenomenon, which is never met with under normal conditions, and hardly ever at any time, made it quite clear that there was excessive shortage of chlorides in the blood. This condition must have been brought about, under the circumstances, by a combination of excessive sweating and drinking of water. Sweating by itself could have no such effect, as follows from the fact that sweat contains only about 0.2 per cent. of chloride, so that sweating by itself would tend to concentrate the chloride in the blood plasma. Mr. Haldane also directed my attention to recent investigations by Rowntree ('American Journal of Physiology,' vol. 59, p. 451 (1922)) on water poisoning produced in animals by ingestion of large quantities of water into the stomach through a tube. The animals showed the severest symptoms, including twitching of muscles, passing on into convulsions. For the theoretical connection between water poisoning due to excessive diffusion pressure of water in the blood, and shortage of salts in the body I may refer to p. 175 of Prof. J. S. Haldane's book on 'Respiration' (1922). It has, moreover, been shown recently by Dr. Priestley ('Journ. of Physiol.,' vol. 55, p. 305 (1921)) that when excess of water is voluntarily drunk the excretion of chlorides by the kidneys falls very markedly, in spite of the enormous excess of urine passed. Miner's cramp, and with it the symptoms of fatigue referred to below, must thus apparently be attributed to water poisoning.

A probable explanation of why the miners drink too much is that thirst depends, to a large extent at least, on dryness of the mouth and throat. In hot and relatively dry air, as at Pendleton, this dryness will tend to be excessive during muscular exertion and the associated increased breathing, and consequently a miner will tend to drink far too much water. The work of Prof. Pembrey and his pupils recently referred to in a preliminary commu-

nication to the Physiological Society, but not yet published in full, shows that with hard work there is complete, or almost complete, cessation of kidney excretion, doubtless owing to the blood being diverted to the muscles. With a big circulation through the skin, as in a hot mine, this effect would probably be produced more easily—that is, with less work. If the kidneys were functioning normally the excess of water would be excreted, but the kidneys are out of action for the reason just mentioned, and the excess of water in the body thus becomes formidable.

On the theory just stated it ought to be possible to prevent the symptoms of water poisoning by supplying the miners with a drink containing just sufficient salt to balance the loss of salt by sweating, and it was decided to experiment upon a few selected colliers by giving them 10 grams of sodium chloride in a gallon of water. The results are extremely interesting and of importance.

The men chosen were sufferers and non-sufferers of good and poor physique. The results are, of course, varied. Some like salt, others dislike it, as is the case with pit ponies. Some pit ponies will lick away in a short time the rock-salt placed in their mangers, whereas others will not touch it. The results obtained are as follows :—

*Case 1.*—E. C., of poor physique, who drinks 8 pints of water during the shift. He has been a frequent sufferer, but since taking salt each day for the past three months has had no sign of cramp. His evidence was as follows : (1) Appetite much improved ; (2) feels quite fresh after a shift's work, where formerly he was obliged to cease work at about 12.30 p.m. each day owing to excessive fatigue ; (3) his life at home was changed from one of laziness and sleep to one full of energy ; (4) in general feels a changed man.

*Case 2.*—T. M., poor physique, but a non-sufferer. Took salt for two weeks only and felt much better for doing so.

*Case 3.*—H. C., a well-built and athletic collier. Took salt for three weeks with the following results : (1) Passed small quantities of urine from 10 to 12 times in the shift instead of twice under normal conditions ; (2) he did not feel anything like so tired after the shift's work ; (3) drank less—about three-quarters of his normal quantity.

*Case 4.*—J. N., of average physique, a non-sufferer. This man only took one-third of the quantity prescribed and was very half-hearted about the experiment. He stated it had not affected him in any way.

*Case 5.*—Of average physique ; a non-sufferer. This man took one-half of the quantity prescribed because the full quantity made him feel sick. He

drinks 5 pints of water per shift. He found that the salt water prevented attacks of dizziness from which he was accustomed to suffer towards the end of a shift's work.

*Case 6.*—J. W., of average physique; non-sufferer. Drinks 5 pints per shift. The salt water did not affect him for better or worse.

*Case 7.*—E. C., of poor physique; sufferer. Was affected similarly to Case 1.

The manager of the mine has discharged all but two of the sufferers so that it was impossible to experiment upon other cramp cases.

Cramp is not confined to miners alone. Ship stokers and iron workers, who also have frequently to work at high temperatures, are liable to it. The writer is obliged to the Assistant Secretary of the Royal Society for calling Dr. Haldane's attention to a graphic description of stokers' cramp in 'The Little Red Captain,' by C. J. Cutcliffe Hyne, of which the following is an extract:—

"Driven half lunatic by the heat and the work, he kept dipping his lips in the water-bucket and drinking heavy draughts. As a consequence that unpoetical complaint cramp in his stomach overtook him at last, and tied him into those ungainly knots of torture which he had so frequently observed upon scientifically in others."

The following description sent to Prof. Haldane by Surgeon-Commander A. Fairley, R.N., may be regarded as typical of a sharp attack of "stokers' cramp":—

"During hard steaming in a hot climate, *e.g.*, Mediterranean in summer, a young stoker is brought up from the boiler room with severe cramp in the muscles of his abdomen and limbs. He walks or is partly assisted to the 'Sick Bay.' The Stoker Petty Officer who brings him has already diagnosed the case and informs you the man is suffering from 'stokers' cramp,' and that his warnings against drinking too much water have been disregarded. Presently the cramp recurs, the abdominal muscles and those of the arms, legs and thighs may together or in turn become bunched up, with hard knots standing out on the muscle due to violent tonic local contraction. The condition is obviously extremely painful, the man writhing and yelling out during the more acute spasms. In such a case massage and warmth alone will not speedily end the attacks, which follow one another almost continuously. A hypodermic of morphia gives him relief, and by his next watch he is well enough to carry on as usual. The condition is not seen so often these days owing to the transition from coal to oil fuel, with its lessened labour and consequently lessened consumption of water while working. The amount of

water drunk is sometimes surprisingly great. During a four-hour watch 10 to 12 pints is not uncommon, but this is unusual among the older and more experienced hands, among whom the condition is seldom met with. As further proof of the condition being not only painful but avoidable, I cannot recall any case of a man having had more than one attack."

Mr. Wallace Thorneycroft, of Glasgow, was good enough to obtain and forward to Dr. Haldane particulars concerning the occurrence of cramp among steel workers exposed to the heat of the furnaces, and the following extracts were taken from several reports submitted by him :--

"In hand-charging days cramp did not often happen with men at work, although men did get cramp when at home. I do not recall half a dozen cases of men having to be taken home owing to cramp."

"Steel melters sweat freely, but experienced hands do not suffer from stomach cramp, as they refrain from drinking when sweating . . . Their practice is to swill out their mouths with water without swallowing it. Inexperienced men, who drink water when in a heated condition, take cramp and become incapacitated for a day or so."

"Cramp, according to our experience, almost entirely depends on the men's own physical condition, and we have heard it freely stated among the men themselves that those who suffer from cramp are those who have had a big supply of alcoholic liquor the night before."

"It is not our experience that excessive sweating produces cramp, and we have never had this fact demonstrated to us in our melting shops, even at the hand-charged furnaces. As a rule this depends very largely on the physical condition of the men themselves. We have been told, however, by experienced and intelligent melters that any distress of this description they experienced arose chiefly from their being called upon unexpectedly to charge their furnaces after having partaken of a very heavy meal . . ."

A common feature of all the reports is that the men drink from 2 to 4 pints of water during work.

The experiments conducted upon A. P. V. and recorded in Part 3 of this paper have a bearing upon this subject. Mr. Veale studied the effects of drinking salt water during work periods, and found that when salt was taken he felt less the fatiguing effects of the work than when abundance of salt-free water was drunk. This bears out the results obtained by the Pendleton colliers who have experimented with the drinking of salt water.

The writer is particularly indebted to Prof. J. S. Haldane for extremely

valuable guidance given throughout, and to Mr. J. B. S. Haldane, Mr. J. Ivon Graham, various mining friends and members of his own staff, for their valuable co-operation. Part of the expenses have been defrayed by the Safety in Mines Research Board.

Further experiments by Mr. A. P. Veale and the writer are at present being carried out on the subjects dealt with in the last two parts of this paper.

*Studies in the Fat Metabolism of the Timothy Grass Bacillus.*

*II.—The Carbon Balance-sheet and Respiratory Quotient.*

By MARJORY STEPHENSON and MARGARET DAMPIER WHETHAM.

(Communicated by Prof. F. Gowland Hopkins, F.R.S. Received June 18, 1923.)

(A Report to the Medical Research Council, from the Biochemical Laboratory, Cambridge.)

It was shown in a previous communication [Stephenson and Whetham, 1922 (1)] that the Timothy Grass Bacillus can be cultivated on a synthetic medium containing potassium phosphate ( $\text{KH}_2\text{PO}_4$ : 0.4 per cent.), and magnesium sulphate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ : 0.07 per cent.), with ammonium phosphate ( $(\text{NH}_4)_2 \cdot \text{HPO}_4$ : 0.4 per cent.) as the sole source of nitrogen, and glucose (1 per cent.) as the sole source of carbon. The cultivation was carried out in Roux bottles, and the hydrogen ion concentration was kept constant at  $\text{P}_\text{H} = 8.0$  by the presence of excess of calcium carbonate. Under these conditions, the glucose was completely used in about 21 days; no breakdown products other than carbon dioxide were found; the protein nitrogen and lipid contents of the bacillus were estimated at intervals and found to attain a maximum immediately before the glucose was used up; the fat of the organism then rapidly fell off, though no decrease was detected in the protein as estimated by Kjeldahl's method. It therefore appeared that while growing in a medium containing a sufficiency of glucose, the organism stored fat, which was oxidised when the glucose was exhausted—the organism living at the expense of its fat and saving its protein.

More recently, somewhat similar work on *Aspergillus niger* has been reported by Terroine, Wurmser and Montané [1922 (2)], who deprived the fully grown fungus of both nitrogenous and carbon-containing foodstuffs, and found a

considerable fall in the percentage content of nitrogen in the organism. Kosinski [1902 (3)] had observed that the respiratory quotient of *A. niger* on a glucose-containing medium is about 1, but falls off to 0.8 during starvation. This result, taken in conjunction with their own, led Terroine, Wurmser, and Montané to the conclusion that *A. niger* uses protein as a reserve material rather than cellulose. No particular observations seem to have been made on the fat content of *A. niger*. The work is not exactly comparable with ours on the Timothy Grass Bacillus, as ammonia was always present in the medium in our experiments.

In order to obtain a clearer view of the physiological economy of the Timothy Grass Bacillus on a synthetic glucose-containing medium, an attempt was made to find in what proportions the carbon of the glucose was finally distributed among (a) the fully grown organism, (b) the carbon dioxide evolved in respiration, (c) the unused breakdown products of the glucose (if any).

Preliminary experiments were made to determine if utilisation of the glucose could be completed without the presence of chalk, which would interfere with the estimation of carbon dioxide. The organism was sown on slopes of glucose tryptic agar at hydrogen ion concentrations varying from  $P_H$  5.6 to 8.0, and it was found that growth occurred from 6.2 to 8.0 with an optimum at about 7.2. Without chalk, the usual inorganic medium had a  $P_H$  of approximately

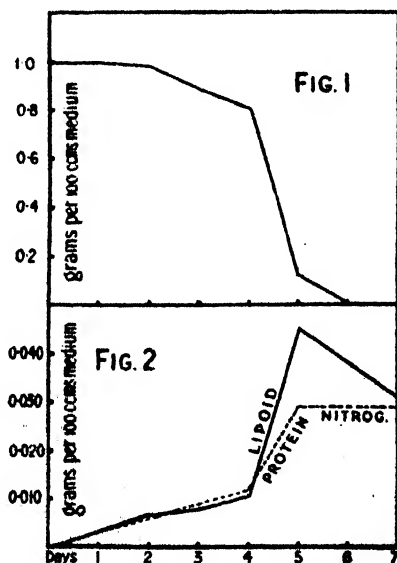


FIG. 1.—Disappearance of glucose.

FIG. 2.—Synthesis of lipoid and protein nitrogen.



7.2 after sterilisation by steaming. Roux bottles containing this, with 1 per cent. glucose, were accordingly sown, and the improvement in conditions resulted in a very rapid growth, the glucose being finished in 5 to 7 days as against 21 days in the presence of chalk. A heavier culture, also, was obtained without chalk, because owing to the shorter period of growth, maintenance requirements were less and more of the food material was available for growth. The usual proportions between lipid and protein in the bacillus were, however, maintained, as was shown by estimations carried out in the manner described in our previous paper and summarised graphically above (figs. 1 and 2). The  $P_H$  fell during the course of the experiment to 6.3. In this as in previous cases we failed to detect the accumulation of any acid products of the breakdown of glucose other than carbon dioxide; the drop in  $P_H$  was attributed (a) to disappearance of ammonia, (b) to carbon dioxide evolved.

In order to determine the carbon balance, the organism was grown on glucose-containing medium in the apparatus shown in fig. 3. The flask A

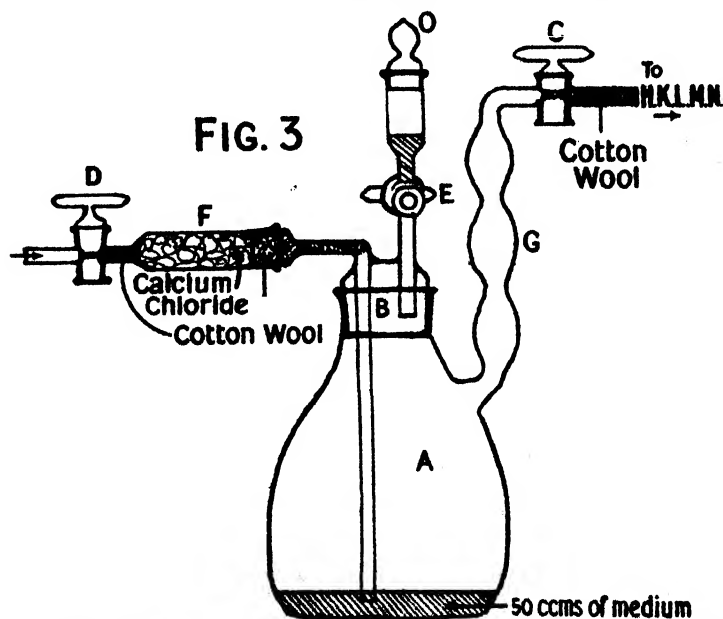


FIG. 3.—Incubation flask for determination of carbon balance and respiratory quotient.

and the stopper segment B, and taps and stopper C, E and O, were separately sterilised in the autoclave, then greased with sterile tap grease, and the parts fitted together, including the calcium chloride guard-tube F, which was not autoclaved. 50 c.c. of medium were run into the flask with a sterile pipette,

and sown with a loopful of culture from a potato slope. The glucose in 50 c.c. of the same medium was estimated by the Wood-Ost reduction method, and checked by the polarimeter. After cooling and weighing, the incubation flask was placed in a water-bath kept at 37° C. by a thermo-regulator, and was connected as shown in the diagram. Dry air, free from carbon dioxide, could be passed by means of an aspirator through F, passing out by the air-condenser tube G. There followed an absorption bottle H, filled with pumice and sulphuric acid, to absorb water from A; sulphuric acid was chosen in case ammonia might also pass over from the medium in A. A second absorption tube K, like the first, was employed to catch any water which might diffuse backwards from a set of potash bulbs L. These, finally, were connected with guard tubes M, of calcium chloride, and N, of soda lime, to prevent back diffusion of water vapour and carbon dioxide.

The incubation flask, prepared as described above, together with the absorption tubes H and K and the potash bulbs L, were cooled and separately weighed and connected up. The carbon dioxide evolved in the incubation flask was driven over into the absorption apparatus by a stream of air once daily, the bulbs of the air condenser G checking the passage of water from A to H. The apparatus was disconnected at intervals (24 hours to 3 days according to the stage reached) and each part was cooled and weighed. The gain in weight of the whole system gave the uptake of oxygen; the gain in weight of K and L gave the output of carbon dioxide. The respiratory exchange over the interval was thus ascertained, and the respiratory quotient calculated. The experiment was ended when the output of carbon dioxide had dropped to so low a value as to indicate that the active life of the organism was over. As a final measure, 1 c.c. of syrupy phosphoric acid was dropped into the incubation flask through the tap E and a stream of air was again passed through, to remove any carbon dioxide held in the medium in chemical combination. K and L were then weighed once more. The contents of A were transferred to a 100 c.c. measuring flask, made up to volume, and the organism filtered off on to a dry filter paper.

A Gooch crucible was lined with filtering asbestos, ignited to redness and cooled; the growth on the filter paper was washed on to the crucible, drained, and dried in a steam oven. The dried bacteria and asbestos were then transferred together to a weighed porcelain boat, and the drying completed *in vacuo* with phosphorus pentoxide at 100° till the weight was constant. The carbon of the bacteria was then estimated in the usual manner in the combustion furnace.

The carbon in the filtrate after removal of the bacteria was very kindly

estimated for us by Mr. N. J. T. M. Needham, using the method recently described by him [1923 (4)]. There was no glucose present in the medium at this time.

A blank experiment to test the apparatus was made by placing 50 c.c. of water and about 0.3 gram. of sodium carbonate in A, connecting up the apparatus and allowing 1 c.c. of syrupy phosphoric acid to fall into A through E. A stream of air was passed through the apparatus for two hours, and the parts were then disconnected and weighed. The figures which follow show the amount of error.

Weights.	Incubation flask.	Absorption Bottles.		Potash bulbs.	Total.
		I.	II.		
Before	111.1080	49.0573	46.0295	105.6300	311.8248
After	110.9723	49.0609	46.0495	105.7381	311.8208

The carbon dioxide originally contained in 50 c.c. of medium was estimated in a second blank experiment, in which 50 c.c. of unsown medium was placed in A, and the carbon dioxide evolved from it by phosphoric acid was swept into the absorption apparatus. The amount was found to be 0.0040 gram., which was consequently deducted from the amount of carbon dioxide obtained from respiration in each experiment.

The carbon balances found in two experiments are given below:—

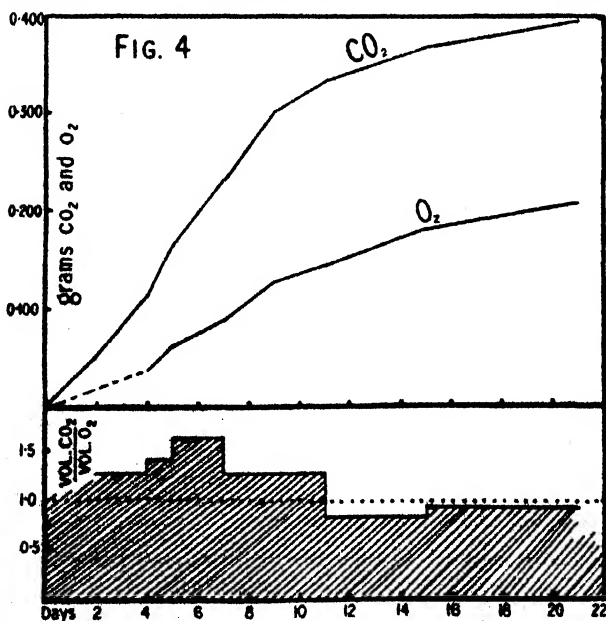
	Experiment 1. 16 days.	Experiment 2. 21 days.
Carbon dioxide from glucose in 50 c.c. of medium .....	0.6590	0.5870
Carbon dioxide from respiration :		
(a) evolved .....	0.3273	0.3986
(b) fixed by medium and obtained by acid .....	0.0023	0.0005
Less carbon dioxide held originally in 50 c.c. of medium (from a blank experiment) .....	0.3296	0.3991
	0.0040	0.0040
	0.3256	0.3951
Carbon dioxide from carbon remaining in filtered medium at the end of the experiment .....	0.0487	0.0416
Carbon dioxide from combustion of the bacillus .....	0.2790	0.1517
	0.6533	0.5884
Error .....	+0.0057	-0.0014
	0.6590    0.6590	0.5870    0.5870

It will be seen that we have succeeded in accounting for all the carbon of the glucose to within 0.5 or 1 per cent. The duration of the experiments (16 and 21 days) is twice to three times as long as in the preliminary experiment in Roux bottles (figs. 1 and 2) ; this is because the growth of the organism is much delayed by the frequent cooling necessary for the intermediate weighings. The final distribution of the carbon of the glucose in the two experiments is recorded in the following table :

Experiment ....	1	2
Duration in days	16	21
Carbon in carbon dioxide of respiration ..	Per cent. 49.41	Per cent. 67.66
Carbon in organism at end of experiment	42.34	25.84
Carbon remaining in medium .....	7.39	7.09

This difference in the proportional distribution is due to the difference in duration of the experiments: the longer the experiment, the more reserve lipid disappears as carbon dioxide, and the less carbon remains in the bacillus.

In figs. 4 and 5 the oxygen uptake and carbon dioxide output of two experiments are plotted against the time, and the change in respiratory quotient



\* FIG. 4.—Above: carbon dioxide output and oxygen uptake. Below: respiratory quotient.

is shown over the same period. During the earlier part of the experiment, when the synthesis of protein and lipoid is taking place and the destruction of sugar

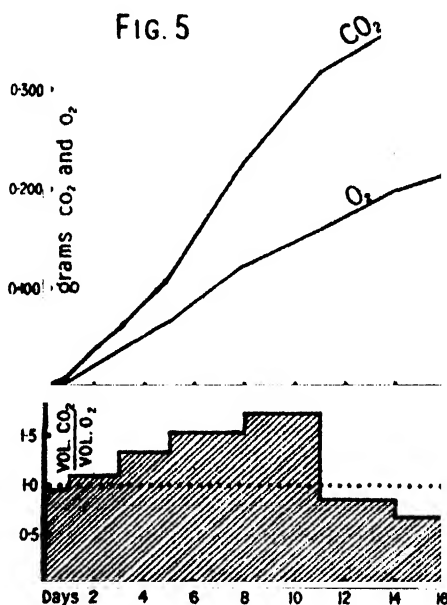


FIG. 5.—Above: carbon dioxide output and oxygen uptake. Below: respiratory quotient.

is proceeding rapidly, the respiratory quotient is high; in the later period, after the sugar has been used up, the output of carbon dioxide falls off and the respiratory quotient drops, indicating that fat is being burnt rather than stored. The change in respiratory quotient thus confirms the change found in the fat content of the organism by analysis (see fig. 2).

Both of us wish to express our thanks for the advice and encouragement received from Professor F. G. Hopkins during the course of these experiments.

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*On the Inheritance of Sinistrality in Limnæa peregra.*

By A. E. BOYCOTT, F.R.S., and C. DRIVER.

(Received June 20, 1923.)

IN 1920, one of the authors commenced breeding experiments with sinistral examples of the normally dextral species *Limnæa peregra*. The animals were kindly given him from an aquarium by Mr. J. W. Taylor, and their sinistral parents had originally come from the pond near Leeds, in which for many years sinistral individuals of this species have been known to occur along with the normal form (8). The animals are sinistral, not only in shell twist but also in the arrangement of the soft parts. The abnormality is very rare, though the species is one of the most abundant fresh-water mollusca.

Four individuals were taken young and put together in two pairs. Large glass jars were used, and in each was placed a piece of water-weed (*Elodea*) from sources known to be free from any possible connection with *Limnæa peregra*. Both pairs produced extensive broods which have now been carried down to the third, and in some few cases the fourth, filial generation.

Owing to the puzzling nature of the early results and the large size of the families, it soon became apparent that the work could not under ordinary circumstances be carried on either under one roof or by one person. Fortunately, assistance was obtained from Mr. L. E. Adams, Mr. R. J. Barker, Mrs. Bateson, Mr. T. H. Burlend, Captain G. C. C. Damant, R.N., Miss Garstang, Mr. W. H. Heathcote, Mr. A. H. Illingworth, Miss Rathbone, Mr. T. H. Riches, Mr. G. C. Robson, Mr. J. J. Simpson, Mr. E. Stainton and Dr. F. M. Turner, each of whom consented to take over both the housing and work in connection with one or more of the families. Without their valuable co-operation it would have been impossible to obtain the mass of data now at our disposal.

In spite of this the position is by no means clear; but as an orderly system of inheritance, differing in certain important particulars from those already known, appears to be emerging from this chaotic mass, it was thought that a brief preliminary statement based on our combined results might now, with advantage, be made.

Certain special difficulties are involved in using this species for genetical enquiry. It is hermaphrodite and in nature seems usually to be cross-fertilised, though in copulation there is not a mutual exchange of spermatozoa as in

the larger land pulmonates. In captivity isolated individuals fairly readily give rise to fertile eggs. We infer that these eggs are the product of self-fertilisation, though this process has not been witnessed by us (5a). These separated parents are, in what follows, spoken of as isolated "singles." Owing to the nature of the characters under examination, it is extremely doubtful whether effective conjugation between individuals showing opposite characters is possible. Further, unlike many land mollusca, sexual maturity is not marked by the cessation of shell growth or the formation of a lip, and individual rates of growth differ considerably under similar conditions. With these difficulties in view it seemed advisable to proceed mainly with single individuals isolated shortly after birth and, where pairs have been used, both individuals of the pair have always shown the same character.

Up to the end of 1922 (including all four generations) 202 broods had been obtained, giving a total of over 16,000 young. Of these broods, 144 were obtained from isolated "singles" and 58 from pairs. Where a pair has been used the brood obtained, although, probably in most cases, contributed to by both individuals, has up to date been regarded as one brood. Further tests are now being made by separating the two individuals of a pair after mating has been observed.

The following types of brood have so far been observed from pairs :—

(1) When both parents have been sinistral in appearance (Sin.) :—

A.	B.	C.	D.
Sin : $\times$ Sin :	Sin : $\times$ Sin :	Sin : $\times$ Sin :	Sin : $\times$ Sin :
All dextral.	3 dextral to 1 sinistral.	1 dextral to 1 sinistral.	All sinistral.

(2) When both parents have been dextral in appearance (Dex.) :—

A.	B.	C.	D.
Dex : $\times$ Dex :	Dex : $\times$ Dex :	(Not yet obtained)	Dex : $\times$ Dex :
All dextral.	3 dextral to 1 sinistral.		All sinistral.

A fifth type of brood (type E) has also been found, at present only in (1), where all the young have the same appearance, with the exception of one or two or rarely three, which have the opposite appearance. As this type of brood

obviously constitutes a special problem, it has not been included here, but will be dealt with later.

In the broods obtained from isolated "singles" we should expect to find the same four types of broods occurring, but although 144 broods have been obtained in this way we find only two of the types represented, namely, types A and D, both of which types may be derived, as in the pairs, from either dextral or sinistral parents. (Type E referred to above is again present, but here it has been derived from dextrals and from sinistrals.)

These results show :—

I.—That, with the exception of the 1 : 1 ratio, which has not yet been obtained from a dextral pair, *pairs of either appearance give the same types of broods.*

II.—That in broods raised from isolated "singles" as well as those raised from pairs, *the appearance of the offspring may be similar or opposite to that of the parent whether this be dextral or sinistral.*

III.—That in broods raised from isolated "singles," "mixed" broods of types B and C are entirely absent.

IV.—That in "mixed" broods, whether the parents are both sinistral or both dextral in appearance, *dextrality in the offspring seems to behave as a simple Mendelian dominant.*

V.—That *the appearance of an individual is no guide to its genetical composition.*

The absence of the "mixed" broods in the case of the isolated "singles" indicates one of several possibilities :

- (i) Parthenogenesis or some abnormal method of fertilisation.
- (ii) Abnormal segregation of factors.
- (iii) The presence of some additional factor or "appearance-determiner."

As a careful scrutiny of this problem has revealed no systems based upon (i) or (ii) which would be at all in accord with our results, we have fallen back on the hypothesis indicated in (iii). This hypothesis suggests the only system we have so far been able to find that is not, to say the least of it, at variance with our present data on any vital point.

As the possibility of comparing directly the proportionate occurrence of individuals of different genetic types within any brood is thus ruled out, the only method of detecting the system of inheritance here in operation seems to be by the comparison of the numerical proportions in which broods of types A and D occur in "first-cousin groups," *i.e.* in a group of broods in which all the broods are derived from a common grandparent. Unfortunately, the



importance of this method was not realised until most of the results now available had been obtained, and, therefore, many of these "first-cousin groups" are of a numerical order such that the addition of one or two more broods would materially alter the ratio.

Nevertheless, there is sufficient evidence to indicate that where the parents of such a group are all of similar *appearance* and have all been treated as isolated "singles," the following *brood ratios* may occur :—

$\alpha$	$\beta$	$\gamma$	$\delta$	$\epsilon$
All broods dextral.	3 broods dextral to 1 sinistral.	1 brood dextral to 1 sinistral.	1 brood dextral to 3 sinistral.	All broods sinistral.

(1) Where the grandparents were a *dextral pair*, giving an *all dextral* brood, "first-cousin groups" of types  $\alpha$  and  $\beta$  have been found, and it is clearly indicated that type  $\gamma$  may also be present.

(2) Where the grandparents were a *dextral pair*, giving an *all sinistral* brood, there is at present insufficient evidence, only one such brood having been carried far enough, and this is complicated by the occurrence of broods of type  $\epsilon$ ; but the evidence seems sufficient to indicate that it constitutes a "first-cousin group" of type  $\delta$ .

(3) Where the grandparents were a *sinistral pair*, giving an *all dextral* brood, again there is insufficient evidence, only one such family having been carried on. The "first-cousin group" obtained may belong either to types  $\alpha$  or  $\beta$ .

(4) Where the grandparents were a *sinistral pair*, giving an *all sinistral* brood, broods have been obtained of type  $\epsilon$ , and also indications are present that broods of types  $\gamma$  and  $\delta$  may occur. One of these groups containing eight families appears to belong to group  $\beta$ , but it seems doubtful whether this is really so.

(5) and (6) where the grandparent was a *dextral isolated "single,"* there is, as yet, no evidence available, as no families of this description have yet reached a second generation.

(7) Where the grandparent was a *sinistral isolated "single"* giving an *all dextral* brood, only one small group is available, but it seems to be a type  $\gamma$  group.

(8) Where the grandparent was a *sinistral isolated "single"* giving an *all sinistral* brood, groups of type  $\epsilon$  are found and there is more or less definite indication that groups of type  $\gamma$  may occur.

It will be seen from these results that in not a few cases the necessary evidence is either absent or not sufficiently strong for a definite statement; but considering the means at our disposal, the nature of the original parents, and the fact that in this preliminary work it was quite impossible to determine which families or groups should be carried on, these gaps cannot altogether be regarded as positive evidence of the non-occurrence of certain types of groups in particular instances.

On the other hand, the results seem to justify the following general conclusions.

I.—That the *appearance* of the grandparents does not affect the types of first-cousin groups that occur among their grandchildren, but the appearance of their children does give an indication of what may or may not occur in these groups. That is, whether the grandparents are dextral or sinistral in appearance, if all their children are dextral in appearance the range of possible groups is from type  $\alpha$  to type  $\gamma$ , whereas if they are all sinistral the range is from type  $\gamma$  to type  $\epsilon$ .

II.—That where the grandparents were a pair three types of first-cousin groups may occur, i.e.  $\alpha$ ,  $\beta$  and  $\gamma$ , or  $\gamma$ ,  $\delta$  and  $\epsilon$ : but where the grandparent was an isolated "single" only two types seem possible, namely,  $\alpha$  and  $\gamma$ , or  $\gamma$  and  $\epsilon$ , groups  $\beta$  and  $\delta$  not being represented.

This leads us to the general proposition upon which the method of inheritance here in operation seems to be based.

III.—That the appearance of the individual is the result of, and is determined by, the product of the parental nuclear composition, but that this individual may carry in itself any of the possible combinations of chromosomes; this nuclear composition gives a fresh product which governs only the appearance of the next generation, within which again any nuclear composition may exist.

The position as regards the occurrence and composition of mixed broods of a Mendelian character is still far from clear. Very few of these broods have been obtained, as incidentally our hypothesis leads us to expect, and these for one reason or another do not provide sufficient evidence to show whether the appearance of the individuals is determined by their own nuclear composition or not. All that is indicated at the present time is, that when two individuals of different nuclear products or appearance-determiners mate, the resulting broods, where both dextrals and sinistrals are present, will show the dextral type behaving as a normal Mendelian dominant, whereas if the appearance-determiners are the same the whole brood will be similar in appearance.



aspect of the problem seems to merit, and is now receiving, special attention, and it is hoped that better results may be obtained.

Among Mollusca generally it is rare to find definite sinistral races of a normally dextral species (or *vice versa*) similar to the case under review (3, 7, 8 and 10), but sporadic sinistrals or sporadic dextrals have been found in many normally dextral or sinistral species (9). Breeding experiments have been attempted with such sporadic sinistrals (5 and 6) and the percentage of their occurrence in natural populations has been examined (1 and 9) ; but no attempt has been made to discover whether the broods containing these sporadic sinistrals occur in similar circumstances and brood-ratios as those examined by us in *L. peregra*. Where as in *Partula* (7) a definite sinistral race has been examined the position seems not dissimilar.

We do not propose at the present time to review the probable causes of inversion (2) nor the occurrence of the problem in other forms of life, beyond indicating that where figures are available for analogous variations (4) in animals or plants they seem to show an underlying similarity of system with the results we have been able to obtain.

The expenses of the enquiry have been partly met from the Graham Research Fund of the University of London.

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*The Effect of a Direct Electric Current of Very Low Intensity  
on the Rate of Growth of the Coleoptile of Barley.*

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(Received May 23, 1923.)

Although many workers have studied the effect of electric currents on the irritable responses of plants there have been only a few scattered observations of the effect of such currents on the rate of growth. A number of the older workers\* investigated the effect on roots of passing electric currents through the soil, but the direct action of the current on the root was not differentiated from the electrolytic action on the soil solution. Later workers appear to have established a response by curvation to weak currents, the so-called galvanotropism of roots.† It has, of course, been known that large currents will bring about the death of cells—no doubt in the main by electrolysis—and that somewhat smaller currents would quickly stop the growth of a stem. Lemström‡ in 1885 and Gassner§ in 1907 each made isolated observations of the effect on seedlings of cereals of a discharge from metallic points fixed above the plants, the points being charged by means of an influence machine. Both report an increase in growth as a result of such treatment, but observations on voltage, strength of current, rate of growth, and the variation between plant and plant are alike wanting. Bose|| has a number of scattered observations, based on single plants, showing that a "tetanising" current lasting a few seconds may increase or decrease temporarily the rate of growth, and that a seedling will exhibit minute changes in the rate of growth when subjected to so-called "wireless" stimulation, which was in reality response to stimulation by a high-frequency current through a wire attached to the plant. These experiments lasted only for a few seconds or at most a few minutes and no measurements of current are given.

Exact knowledge of the direct effect of electric currents on the rate of growth

\* W. Pfeffer, 'Plant Physiology' (Oxford), vol. II, p. 107 (1903).

† W. Rothert, 'Zeit. Allg. Physiol.', vol. VII, pp. 142-164 (1907).

‡ S. Lemström, 'Electricity in Agriculture and Horticulture' (London) (1904).

§ G. Gassner, 'Ber. Deut. Bot. Ges.', vol. XXV, pp. 26-38 (1907).

|| J. C. Bose, "Plant Response (London)," 127, 436, 1906, 'Transactions of the Bose Research Institute (Calcutta)', vol. II, p. 421 (1919).

of actively developing organs was thus practically wanting. Since there was this gap in our knowledge, and the authors had been engaged on studying the effect of a high-tension electric discharge on the dry weight and grain production of plants, it was determined to make a careful study of the growth-reactions of plants to electric currents which continue over a considerable period. In such a study, carefully controlled conditions and accurately measured currents must be employed, and full consideration must also be paid to the variation in the response of individual plants. Accordingly, experiments were begun in 1918 on the coleoptile (sheathed plumule or young stem) of the seedling of barley (*Hordeum vulgare*). In all the experiments no electrodes in contact with the plant were used, but the ionization current from a highly charged point placed at a little distance above the plant was employed. The progress of the work has been delayed owing to the fact that the observations could only be made during half the year, and also owing to the great variability in the rate of growth of individual plants, so that for a trustworthy mean result numerous observations were required.

#### *Experimental Technique.*

The method was to grow seedlings in a culture solution in a dark room kept at constant temperature, and to measure the rate of growth every 15 minutes, the plants being exposed to weak red light only, for the brief period necessary to take the reading. Measurements were made over a period of eight hours, two seedlings at a time being under observation. The current was obtained by means of the glow discharge from a metallic point charged to a high voltage and placed vertically above the coleoptile. The details of the technique finally employed are described below.

*Treatment of Seedlings.*—A "pure line" of barley (Goldthorpe) obtained from the Research Department of the Olympia Agricultural Company was employed. The grains were carefully graded as to weight and germinated between filter papers in petri dishes in the dark room. On the afternoon of the second day of germination, when most of the coleoptiles were beginning to show through the glumes, eight seedlings were selected. These were set up in the dark room in nutrient solution in small tubes (figs. 1 and 2) carrying at the base a platinum wire, fused through the glass, by means of which the current passing from the seedling could be led to a galvanometer; care was taken that the base of the coleoptile was not subjected to pressure. The following morning the two seedlings with the straightest coleoptiles were selected and placed as shown in fig. 1, in an inner chamber standing inside

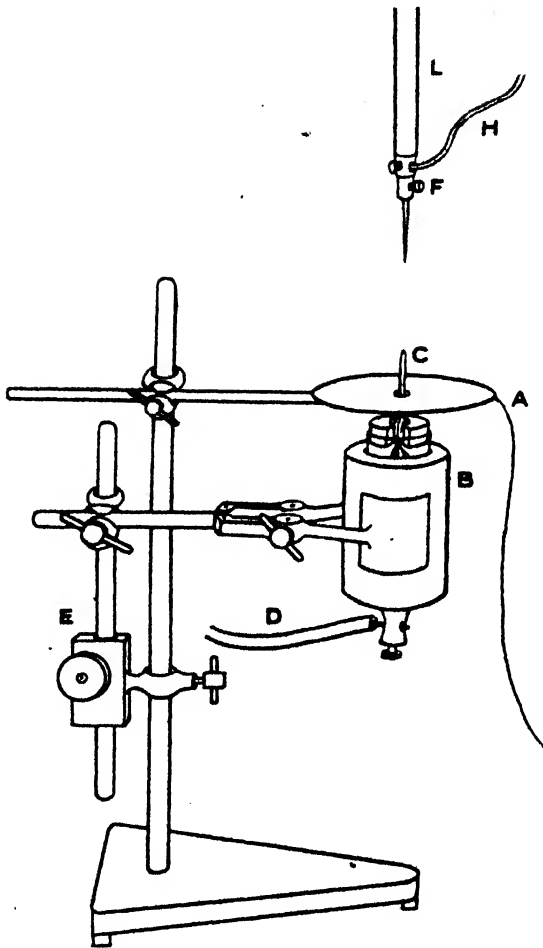


FIG. 1.



Fig. 2.

FIG. 1.—Diagram of apparatus with barley seedling in position. A, guard ring with earth wire; B, paraffin wax cup, holding glass tube with seedling; C, coleoptile; D, lead to galvanometer; E, rack and pinion movement for raising and lowering the coleoptile; F, brass mount with discharging needle; H, high-tension lead; L, insulating rod.

FIG. 2.—Section through B of fig. 1 showing method of fixing the seedling in the cork. B, paraffin wax cup holding glass tube with culture solution; P, platinum wire fused through test-tube into tube of mercury (M) into which the binding screw (N) is sealed.

the dark room. Care was taken to avoid shaking the coleoptile or allowing it to come in contact with any object, since shaking was found to affect markedly its rate of growth.

*Measurement of Rate of Growth.*—After the two seedlings were in position the door of the inner chamber was closed and two horizontal microscopes reading to 0.01 mm. were placed in position, so that the coleoptiles could be viewed through two apertures in the doors of the chamber against a background illuminated by weak red light. After the lapse of half an hour, readings of the rate of growth were begun and continued every 15 minutes throughout the duration of the experiment.

*Control of Temperature.*—The temperature of the dark room was kept constant at 19° C. or 20° C. by means of a regulator, consisting of many parallel turns of thin glass tubing holding mercury. The movements of the mercury column controlled a weak electric current through a platinum wire fixed above the column. The current actuated a relay, which in turn controlled the main current through a small electric, non-luminous heater over which a current of air from an electric fan continually passed. This arrangement was very effective, the current starting and stopping every few minutes and the temperature of the dark room itself being regulated to 0.1° C., while the temperature of the inner chamber containing the seedlings rarely varied more than 0.04° C, during an experiment, and would often remain constant for many hours within a range of 0.01° C.

*Supply and Measurement of Current, etc.*

Alternating current at about 150 volts, 50 cycles, was obtained from the ordinary laboratory supply (220 volts D.C.) by the use of a small rotary converter. With the help of a wax-impregnated 50,000 volt transformer the voltage could be raised as required, a current at about 10,000 volts (crest value) being usually employed, the voltage being measured by the spark-gap between needle-points; rectification was obtained by means of a Lodge valve. Thus the current was uni-directional, with 50 pulses per second. The current from the charged point, fixed at a height of about 20 cm. above the plant, after passing through the coleoptile and seedling was led off from the platinum wire at the base of the tube holding the culture solution (figs. 1 and 2) to a galvanometer, placed some yards away in another room so that it should not be affected by the high-tension current. As the current to be measured was exceedingly small a very sensitive galvanometer was necessary. An instrument made by R. W. Paul, of the moving coil type, with a resistance of 2180 ohms and giving a deflection of 20,000 mm. per microampere, was employed. In order to ensure that the galvanometer received only the current passing through the plant, the coleoptile was surrounded



with an "earthed" guard-ring as shown in the illustration (fig. 1). The charged needles were held in long, weighted, "bakelite" rods (fig. 3) suspended

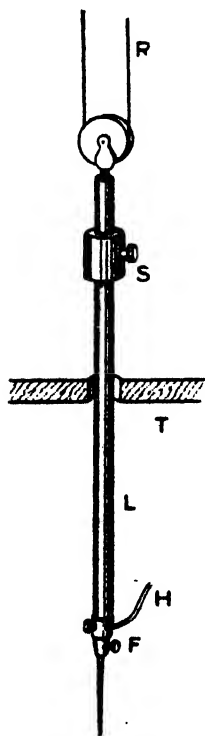


FIG. 3.—Illustration of the method of mounting and controlling the needle. F, brass mount with needle; H, high-tension lead; L, insulating (bakelite) rod; T, top of inner chamber; S, brass collar to prevent the needle being lowered within sparking distance of the coleoptile; R, fine cord passing to the controlling winch (in the galvanometer room) by which the needle can be raised or lowered.

from cords which were carried to the galvanometer room. By means of these and a small winch the needle-point could be raised or lowered while observing the deflection of the galvanometer; the strength of current employed was thus regulated with great precision. By controlling the height of the needle-point the current for all three sets was kept to a strength of about  $0.5 \times 10^{-10}$  amp.\* The coleoptile measures about  $0.013 \text{ cm}^2$  in cross-section.

#### *Method of Stating Results.*

As the rate of growth of individual coleoptiles is very variable, and the normal rate increases throughout the period of observation, it seems most satisfactory to express the results not as absolute, but as relative, hourly rates; the rate of growth of each coleoptile for the hour immediately preceding the experimental period of five hours was, therefore, taken as the standard rate for that coleoptile and expressed as 100. In physiological work of this kind, in which the means of a number of varying rates are the basis of comparison, a knowledge of the extent of the experimental error is all important, since on that depends the significance of the results. The probable error of the means have, therefore, been computed and the number of observations multiplied so as to reduce these errors to a comparatively low level.

#### *Experimental Results.*

Four series of experiments were made in which the hourly rates of growth during a period of five hours were compared with the rate of growth (expressed as 100) for

\* As the galvanometer was being used near the limit of its sensitiveness this reading is probably only accurate within 25 per cent.

the hour immediately preceding the five-hour period. Apart from the control plants, in which no electrification was given, experiments were made with (1) plants in which the discharge was applied for *three* hours with the point *positively* charged, (2) plants similarly treated but for *one* hour only, (3) plants in which the current was applied for *three* hours but with the point *negatively* charged.

Table I.—Barley in nutrient solution. 3.2.22. Set up 10.15 a.m. Discharge positive. Point, 20 cm. above plant A, and 19 cm. above plant B. Coleoptile 8 mm. long. Spark gap 9 mm.

Time.	Micrometer Readings.	Hourly growth-rate.	Ratio of hourly growth-rates.	Current (amp. $\times 10^{-10}$ ).	Micrometer Readings.	Hourly growth-rate.	Ratio of hourly growth-rates.	Current (amp. $\times 10^{-10}$ ).	Temp. $^{\circ}$ C.	
Seedling A.					Seedling B.					
12.0	3.11				3.11				20.83	
12.15	3.39				3.35				20.82	
12.30	3.62				3.57				20.82	
12.45	3.81				3.78				20.82	
1.0	4.10	0.99	100		3.99	0.88	100		20.82	
1.15	4.40			0.5	4.20			0.5	20.80	Discharge started at 1.0.
1.30	4.61				4.41				20.79	
1.45	4.84				4.65				20.78	
2.00	5.14	1.04	105.0		4.90	0.91	103.4		20.79	
2.15	5.50			0.5	5.19			0.5	20.79	Discharge stopped at 4.0.
2.30	5.71				5.40				20.79	
2.45	5.93				5.65				20.78	
3.0	6.30	1.16	117.1		5.91	1.01	114.7		20.78	
3.15	6.67			0.5	6.20			0.5	20.79	Discharge stopped at 4.0.
3.30	6.90				6.44				20.79	
3.45	7.19				6.71				20.79	
4.0	7.56	1.26	127.2	0.5	7.01	1.10	125.0	0.5	20.79	
4.15	7.89 (3.00)				7.30				20.79	
4.30	3.25				7.58				20.79	
4.45	3.50				7.87 (3.00)				20.79	
5.0	3.90	1.23	124.4		3.30	1.16	131.8		20.79	
5.15	4.29				3.60				20.79	
5.30	4.72				3.90				20.79	
5.40	4.85				4.19				20.79	
6.0	5.22	1.32	133.3		4.51	1.21	137.5		20.79	

In the control experiments 41 plants were investigated, part in October, 1921, and part in March, 1922. The differences between the two sets were found to fall within the probable error; in the other three sets the means are based on observation of 30, 20 and 21 plants respectively. The results of one day's experiments are given in Table I, and the mean results of all the experiments in Table II and are also shown graphically in fig. 4.

Table II.—Mean Hourly Rates of Growth of Coleoptile of Barley expressed as Percentages of the Standard Rate.\* Current per plant =  $0.5 \times 10^{-10}$  amp.

Hours.	Control (41 plants).	I.—Positive Discharge 3 hours (30 plants).	II.—Positive Discharge 1 hour (20 plants).	III.—Negative Discharge 3 hours (21 plants).
1 .....	104.27 $\pm$ 0.81	108.92 $\pm$ 0.88	108.74 $\pm$ 0.85	108.23 $\pm$ 1.18
2 .....	114.60 $\pm$ 1.09	120.46 $\pm$ 1.53	121.29 $\pm$ 1.20	117.60 $\pm$ 1.35
3 .....	121.34 $\pm$ 1.22	128.87 $\pm$ 1.52	127.90 $\pm$ 1.78	123.76 $\pm$ 1.30
4 .....	122.10 $\pm$ 1.19	130.32 $\pm$ 1.92	132.11 $\pm$ 1.70	128.34 $\pm$ 1.43
5 .....	124.63 $\pm$ 1.42	135.33 $\pm$ 2.04	140.31 $\pm$ 2.20	133.04 $\pm$ 1.66

\* The rate of growth for the hour previous to the start of the experiment is taken as the standard rate.

The rates of growth for the periods during which electrification was given are shown in block type.

It is to be noted that in the controls, as already stated, the rate of growth of the plants is increasing throughout the period of observation, the rate for the first hour being 4 per cent. above the standard rate of the previous hour, and the fifth hour nearly 25 per cent. above that standard rate. It is also evident from a comparison of the three electrified sets with the control set that the passage of the current is associated with a definite increase in the rate of growth, the augmentation of growth in sets I and II rising steadily from hour to hour. The effect of the current is, however, more clearly shown by the differences between the hourly rates of the control plants and those of the three sets subjected to electrification. These differences with their probable errors are set out in Table III.

The marked effect of the "downward" current (*i.e.* with the discharging point positive) in stimulating growth is well seen in experimental sets I and II of Table III, where the increases in the rate of growth produced by the current rise gradually with time and are in all cases over three times the probable error, and in some cases exceed four and five times that error.\* In set I the

\* Such a series of results as that set out in Table III provides, of course, overwhelming evidence of the effect of these minute discharges on the rate of growth; the odds against the accidental occurrence of even the first four results of set I are over 10,000 to 1.

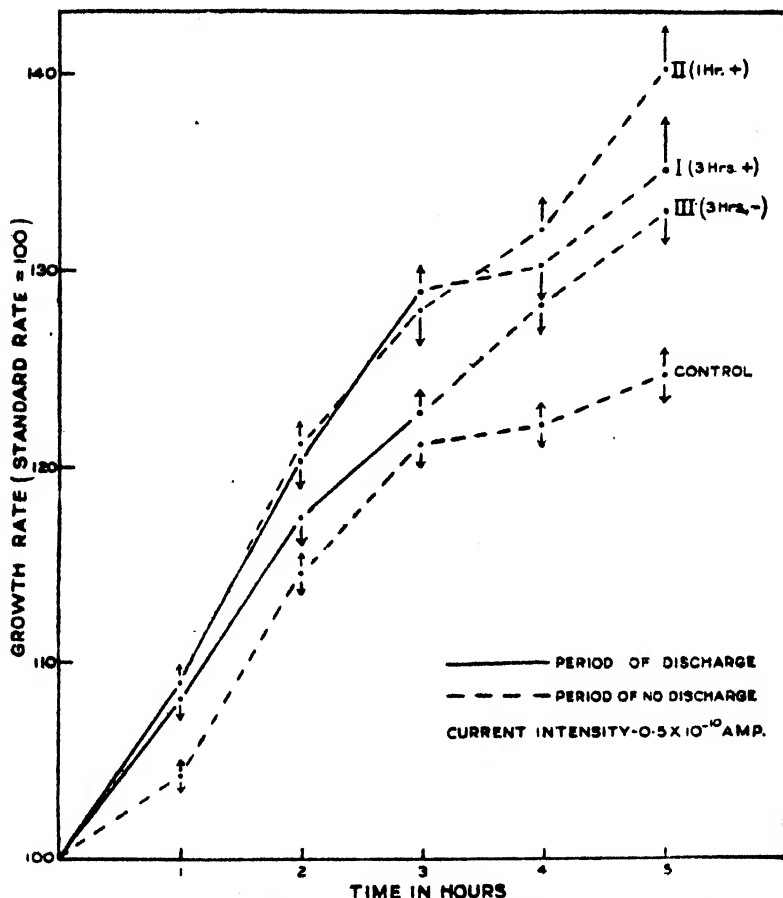


FIG. 4.—Graph showing the mean hourly rates of growth of control coleoptiles and of coleoptiles exposed (I) to a positive discharge for the first three hours, (II) to a positive discharge for the first hour, (III) to a negative discharge for the first three hours. The growth rates are expressed as percentages of the "standard rate." The length of the arrows shows the size of the probable error of the various means; in most cases it is shown in one direction only.

increase in the first three hours is, respectively, 4.65 per cent., 5.86 per cent. and 7.53 per cent. of the standard rate above that of the unelectrified control plants; this corresponds to 4.45 per cent., 5.11 per cent. and 6.36 per cent. respectively of the actual average rate of the controls. In set II the increased growth during the period of electrification is 4.47 per cent., differing from set I only by an amount considerably less than the probable error.

In both sets it is to be noted that the increased rate not only continues after the cessation of the discharge but even grows greater. There is thus a

Table III.—Percentage Differences between Mean Hourly Rates of Growth of Coleoptiles of Electrified Plants and Control Plants. The differences are expressed as percentages of the "standard rate."

Current per plant =  $0.5 \times 10^{-10}$  amp.

Hours.	I.—Positive Discharge 3 hours.	II.—Positive Discharge 1 hour.	III.—Negative Discharge 3 hours.
1 .....	+ 4.65 ± 1.19	+ 4.47 ± 1.17	+ 3.96 ± 1.39
2 .....	+ 5.86 ± 1.88	+ 6.69 ± 1.62	+ 3.00 ± 1.74
3 .....	+ 7.53 ± 1.95	+ 6.56 ± 2.13	+ 1.42 ± 1.71
4 .....	+ 8.22 ± 2.26	+ 10.01 ± 2.08	+ 6.24 ± 1.86
5 .....	+ 10.70 ± 3.00	+ 15.68 ± 2.62	+ 8.41 ± 2.18

The results for the periods of electrification are shown in block type.

very remarkable *after-effect* of the discharge, which is to be observed for two hours in set I and for four hours in set II. It is still increasing up to the end of the period of observations, reaching in the fifth hour of set II 15.65 per cent. of the standard rate (= 12.58 per cent. in excess of the rate of the control coleoptiles). A comparison of the growth-rates for the fourth and fifth hours of sets I and III shows also that the after-effect in these hours is greater with a short period than with a longer period of discharge, the excess in the fifth hour being nearly 50 per cent. greater after one hour's electrification than after three hours. The duration of the after-effect has not yet been ascertained.

The effect of an "upward" current, with the discharging point negatively charged (set III), is, for the first hour, slightly stimulating, though possibly less so than that of the "downward" current; for the second hour the effect is very slight, and for the third hour the effect is probably *nil*, the difference from the control plants being less than the probable error. The steady fall over the three hours in the stimulating effect suggests that if this negative current had been continued for a longer period a retardation of growth would have been produced. For the two hours, however, after the cessation of the current the rate of growth compared with that of the controls increases again so that here also there is a well-marked *after-effect*. This after-effect, however, is distinctly less than that resulting from a "downward" current given for the same period (set I), and very much less than the after-effect obtained in set II.

*Effect of other Factors Associated with the Discharge.*

In a high-tension discharge of this type the ionic current is associated with gaseous products, such as oxides of nitrogen, and also with a mass movement of air, the so called "electric wind." The question, therefore, arises as to whether the increased rate of growth brought about by the discharge may not be due, solely or in part, to the action of factors other than the ionic current. The striking fact, however, that the direction of the current so markedly affects both the degree of direct stimulation and also the after-effect, is almost by itself sufficient to exclude the view that these other two factors are of importance. As far as movement of the air is concerned it seems unlikely that such movement would accelerate the rate of growth; a retardation rather would be expected. To submit the problem of the effect of the other factors of the discharge to experimental test use was made of the observations that discharges of higher intensity, such as those in which a current of the order of a microampere passes through the plant, bring about a marked retardation of growth.

A series of experiments was conducted in which the plant was subjected

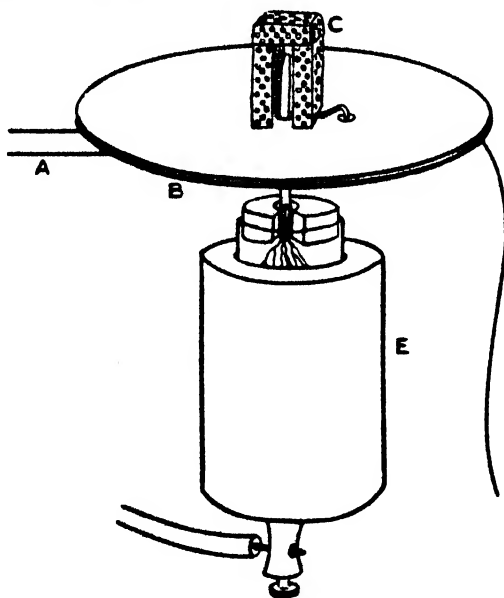


FIG. 5.—Diagram of apparatus employed to screen the coleoptile from the current while subjecting it to the action of the gaseous products of the discharge. A, support; B, metallic guard ring bearing mica disc; C, perforated screen of platinum foil which stands over the coleoptile and is earthed to the guard ring through a hole in the mica disc; D, wire to "earth"; E, paraffin cup as in figs. 1 and 2.

to a discharge of an intensity sufficient to retard markedly the rate of growth, but the current was deflected to an "earthed" screen of metal gauze, while the coleoptile remained exposed in full to the gaseous products of the discharge and also to air movement brought about by the discharge. In these experiments a piece of *perforated* platinum foil, shaped as shown in fig. 5, was placed on a mica disc covering the upper surface of the guard ring so that it stood over the top of the coleoptile without, however, touching it. The screen of platinum foil was "earthed" to the metal guard ring through a hole in the mica disc. A current of a few microamps. was then sent through the system, but only a small part reached the plant, the main current passing through the screen and guard ring to earth. The current actually passing through the coleoptile was, however, larger than that employed in the previous experiments. With the screen in position, the plant was exposed to the gaseous products of the discharge, and in part to the "electric wind,"

Table IV.—Growth of Screened and Unscreened Coleoptile exposed to Large Discharges.

	Hourly Rate of Growth compared with Standard Rate (100).		Current through Plant.	Total Current.
	Screened.	Unscreened.	Amp. $\times 10^{-6}$ .	
1 .....	106.06		0.01	0.26
		60.00	0.11	0.20
	95.24		0.003	0.23
		73.33	0.11	0.20
2 .....	88.43		0.009	0.26
		54.54	0.12	0.22
3 .....	98.66		0.006	0.26
		72.66	0.14	0.25
4 .....	91.93		0.001	0.25
		66.12	0.16	0.25
5 .....	81.10		0.007	0.50
		61.41	0.40	0.50
6 .....	97.60		0.01	0.51
		48.00	0.40	0.50
7 .....	107.14		0.01	0.51
		90.00	0.40	0.50
8 .....	100.16		0.008	0.51
		81.46	0.40	0.50

Voltage, 17,000 (crest value). Air gap, 3 cm.

yet the retardation of growth was *nil* or only slight. When, however, with the screen removed, the same or a slightly smaller current was sent through the system, the coleoptile received the main portion and showed a marked retardation; the production of gaseous products, however, was not less, and the electric wind was probably somewhat increased. The results of nine experiments with eight different coleoptiles are shown in Table IV, and the result of one experiment is shown graphically in fig. 6.

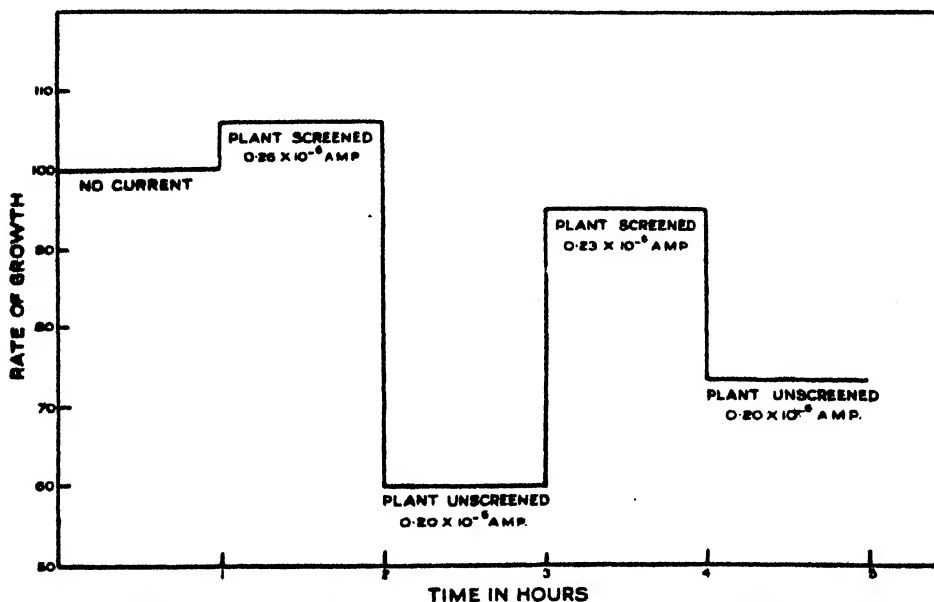


FIG. 6.—The effect of large currents on the rate of growth of screened and unscreened coleoptiles. In the second and fourth hours the screened coleoptile received only a small current and the retardation is nil or slight. In the third and fifth hours the unscreened coleoptile received the whole current and the growth is markedly retarded.

From this set of experiments one may conclude that the gaseous products of the discharge play no part in the retardation of growth brought about by a strong electric discharge to the coleoptile. Since, as is to be expected, acceleration and retardation are parts of a perfectly continuous process depending on the intensity of the discharge, the conclusion can also be drawn that the gaseous products of the air—other than of course the ions of the air—play little or no part in the stimulation of growth here described. The effect of air movement is not completely excluded, but since no air movement can be felt by the back of the hand when the charged point is at the distance at which



increased growth is produced in the coleoptile,\* the effect, if any, must be very slight, and, as already indicated, would probably be one of retardation. Since the action of the gaseous products is excluded and the effect of movement of the air unlikely, and as furthermore the direction of the current influences so markedly the degree of physiological response, there is a very clear indication that the stimulation of growth is due to the ionic current passing to the plant.

#### *Discussion of Results.*

That such small discharges as these, in which the direct current passing through the plant is of the order of  $0.5 \times 10^{-10}$  ampere, should have a stimulating effect on the growth of the coleoptile of barley, and that such stimulation should continue with increasing effect for at least several hours, is certainly very striking. The fact that the stimulating action continues after the cessation of the current—the after-effect being more pronounced than the direct effect, and being still more pronounced with a short period (one hour) of discharge than with a long (three hours) period is also of great interest. A number of questions naturally arise, such as that of the period during which the discharge will continue to give a direct stimulation, the degree of increased growth which such stimulation can bring about, the height to which the after-effect will rise, and the time during which it will continue. There is also the problem of the period of stimulation necessary to produce the maximum after-effect, and also that of the effect of different frequencies and of different strengths of current; some of these are now under investigation.

The question may also be asked as to whether the observed effect on growth is due to the passage of current through the coleoptile† or to some special action, possibly surface action, of the ionisation current.‡ The first supposition is certainly the more probable, since marked irritable responses to ordinary electric currents of higher intensity are well known. The question would be settled if a similar effect on the growth-rate could be obtained with low-voltage currents of the same order. Rapidly growing organs, however, are usually very sensitive to contact, as has already been stated, so that it would be difficult to obtain normal growth over any considerable period when using electrodes in contact with the plant.

\* Cook ('Philosophical Magazine,' vol. XLVII, p. 44 (1899) found that the electric wind was not sufficient to move a lightly poised paper vane at a distance greater than 32 cm. with a voltage of 25,000 and the discharge positive.

† It would seem unlikely that the electric field apart from the current has any effect on the rate of growth of the coleoptile.

‡ It has been claimed that ionised air has a special physiological action on plants.

In view of our comparative ignorance of the physiology of growth, it would seem idle to speculate at present on the nature of the phenomena here described. A reaction which is characterised by an after-effect not only greater than the direct effect, but greater with a short period than with a long period of stimulation—a reaction, moreover, in which the direction of the current so markedly affects the degree of the response—is certainly one not easy of interpretation in the present state of our knowledge of cell-dynamics.

### Summary.

The rate of growth of the coleoptile (sheathed stem) of the barley seedling is increased when it is exposed for a period of one or three hours to an electric discharge from a point charged *positively* to about 10,000 volts (crest value), and placed at such a height (about 2 cm.) above the coleoptile that a current of the order of  $0.5 \times 10^{-10}$  ampere passes through it.

The increase in the rate of growth reaches  $4.65 \pm 1.19$  per cent. of the "standard" rate (4 per cent. of the normal rate) in the first hour, and if the current is continued for another two hours the effect goes on increasing, showing in the third hour an excess of  $7.53 \pm 1.95$  per cent. of the "standard" rate (5 per cent. of the normal rate).

When the current is stopped there is a very remarkable *after-effect*, for the rate of growth continues to rise for at least four hours after the cessation of the discharge; this after-effect is greater than the direct effect, and may result in the fifth hour in an increase in growth of  $15.68 \pm 2.62$  per cent. of the "standard" rate above that of the control plants (equivalent to 12.58 per cent. of the actual rate of the control plants).

The after-effect, as measured by the increase in the rate of growth in the fifth hour from the start of the discharge, is greater with the short discharge-period of one hour than with the longer discharge-period of three hours.

When the point is *negatively* charged and a current of the same intensity passed through the seedling for three hours the rate of growth increases during the first hour, but the increase, instead of becoming greater with time as with a current in the other direction, becomes less, so that in the third hour the rate is little, if at all, above the normal. When after three hours the current is stopped, an after-effect, exhibiting itself as before in an increased rate of growth, is to be observed; the after-effect for the two hours during which it was followed is markedly less than that shown when the current is in the other direction.

Experimental evidence is advanced for the view that the gaseous products

of the discharge and the "electric wind" play little or no part in the stimulation of growth observed. The view that the current alone is of importance in the reaction is also supported by the fact that its direction exerts such a marked influence on the degree of stimulation.

This investigation has been undertaken in connexion with experimental work for the Electro-Culture Committee of the Ministry of Agriculture and Fisheries. It has been carried out with the aid of special grants from the Development Commission. We have to thank Prof. G. W. O. Howe for his kind help in some electrical matters.

### *The Significance of an Achondroplasia-like Condition met with in Cattle.*

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(Communicated by Sir Arthur Keith, F.R.S. Received April 18, 1923.)

[PLATES 12-15.]

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#### *I.—The Dexter.*

The Dexter is a breed of the smallest cattle in Great Britain. Formerly it was indigenous to the south and south-western districts of Ireland, but of late years it has become increasingly popular in England.

Its general appearance, as defined in the terms of the standard of excellence laid down in the Kerry and Dexter Herdbook, is as follows :—

*Head* short and broad, with great width between the eyes and tapering gracefully towards the muzzle which should be large with wide distended nostrils. Eyes bright, prominent, and of a kind and placid expression. Neck short, thick, and deep, and well set into the shoulders, which, when viewed in front, should be wide, showing thickness through the heart, the breast coming well forward.

*Horns*.—These should be short and moderately thick springing from the head with an inward and slightly upward curve.

*Body*.—Shoulders of medium thickness, full and well filled in behind; hips wide, quarters thick and deep and well-sprung, flat and wide across the loins, well ribbed-up, straight underline, udder well forward and broad behind, with well placed teats of moderate size; legs short (especially from knee to fetlock), strong, and well placed under the body, which should be as close to the ground as possible. Tail well set on and level with the back.

*Skin*.—The skin should be soft and mellow, and handle well, not too thin; hair fine, plentiful and silky.

*Coat colour*.—Bulls, whole black or whole red (the two colours being of equal merit). A little white on the organs of generation not to disqualify an animal which answers all other essentials of this standard description.

Cows, black or red (the two colours being of equal merit). White on udder and the extension of white on udder slightly along inside of flank or underside of the belly, or white on tassel of tail, may be allowed on an animal which answers all other essentials of this standard description.

*Weight*.—Bulls should not exceed 900 lbs., live weight, when in breeding condition. Cows should not exceed 800 lbs.

In view of that which is to follow, it is desired to call attention to these two characters of the ideal Dexter—brachycephaly and micromelia.

Two undesirable characters are encountered occasionally, "bad tail-head," the tail not being terminal but seeming to take origin further forward along the back and arching upwards and backwards, and a combination of bent forelegs with inwardly turned hoofs. "Its toes turn in after a peculiar fashion, and it tends to walk over the outer digits, especially in the case of the hind feet."

The history of the Dexter is wrapped up in that of the Kerry, and like that of almost every breed is befogged by anecdote and speculation. It is generally accepted that the Dexter is an offshoot of the Kerry; it is certain that the breed has arisen out of the old-fashioned Kerry stock as a result of an outcross.

The native ancestral stock from which the modern Kerry has been developed was a black-coated race, being of the same stock as the native Celtic cattle of Great Britain. The modern Kerry is all that remains of the race which in former days was to be found throughout the whole length and breadth of Ireland. There are historical records of the importation of Longhorns, Shorthorns, Herefords, and Devons into Ireland, and by the middle of the nineteenth century the old native race as such had become almost entirely replaced by imported stock, and was extinct in all parts save in Kerry and Donegal.

Wilson has given an exhaustive survey of the literature which bears upon the origin of the Dexter breed and upon the manner in which it got its name. He concluded that the early records of the Dexter herd are unreliable, and that a better idea of its ancestry can be derived from the genetical analysis of the modern Dexter.

## *II.—The Genetic Constitution of the Modern Dexter.*

A statistical study of the results of the mating of Dexter with Dexter shows that four classes of calves are produced—Black Dexter-type, Red Dexter-type, Black Kerry-type and Red Kerry-type. By Dexter-type is meant a relatively massive head, a stout body and short limbs; by Kerry-type is meant a relatively slender head, a slim body and long limbs. Of the four classes, the Black Dexter-type is by far the most common, the Red Kerry-type the least common, while the other two occur in more or less equal numbers.

The appearance of four phenotypes in such proportions suggests at once that the Dexter is, itself, to be regarded as a Mendelian di-hybrid, and that its parental stocks differed one from the other at least in respect of two pairs of contrasted characters, coat colour and bodily conformation. The contrasted characters concerned were black and red coat colours, and Dexter-type and Kerry-type bodily conformations. The old type Kerry, black, slender and long-limbed could have been one of the parental stocks. If this were the case, then the other must have been a red, stout, short-limbed stock.

Wilson brings forward considerable evidence to show that such an animal was imported into Kerry during the formative period of the Dexter, and that it was crossed with the native Kerry stock. He quotes Wakesfield as saying that: "In the South I met with some persons who had imported Devon cattle; Lords Bantry, Shannon and Doneraile, Mr. Hyde and others possess

considerable numbers of them." Wilson also points out that in the seventeenth and eighteenth centuries many English emigrants, sailing from Bristol Channel ports, settled in Kerry and West Cork, bringing with them their own red cattle.

Wilson sums up the evidence thus: "The probability, therefore, that Dexter cattle are descended from Black Kerries and Red cattle of Devon type is very high; and if further proof were wanted, it can be found by setting a Red Dexter cow side by side with a Red Devon." The modern Red Dexter is a mottled red, showing delicate blackish tracers on the red ground; this same mottling is seen in the modern Red Devon.

It is indeed probable that the Dexter had its origin in the mating of Black Kerry and Red Devon. It would seem to be a simple matter to put this suggestion to the test of direct experiment by mating a Kerry and a Devon. But the modern Kerry is not the Kerry of the time when the Dexter was produced, nor is the modern Devon genetically the same as the Devon taken to Ireland by the English emigrants.

Supposing, as stated, that the original parents, Kerry and Devon respectively, introduced the dominant factors B and S to denote black coat colour and short-legged bodily conformation, it is easy to see that the result of backcrossing to either type of parent would give 50 per cent. Black Dexter type, while inter-breeding between the heterozygous Black Dexters would give Black Dexter offspring in the proportion 9/16. These Dexters would be of four types according as they were homozygous for both dominant factors, heterozygous for both, or homozygous for one and heterozygous for the other. As soon as the picture of the ideal Dexter took shape in the breeders' mind, the production of a pure strain homozygous for both factors followed automatically by the elimination of the Red Kerry and Red Dexter off-types.

### *III.—The Monstrous Calf of the Dexter.*

As time went on, it may be assumed, the matings became more and more confined to those between Black Dexter-type and Black Dexter-type, and that the phenotypic selection which was thus practised was really meant to isolate the genotype BBSS.

Slowly it came to be recognised that this phenotypic selection resulted in the production of a deformed and still-born calf; that, as the mating of Dexter-type and Dexter-type became more common, the proportion of these monstrous calves increased. Ultimately, it was accepted that with such

matings a proportion of still-born monstrous calves was to be expected. In 1919, the society formed in 1917 to promote the interests of Kerry and Dexter cattle breeding in Ireland, decided to change its name to "The Kerry Cattle Society of Ireland." "This alteration was deemed advisable as herds of pedigree Dexter cattle have practically ceased to exist in Ireland owing to the difficulty of breeding these cattle pure. It is the experience of Irish breeders that when Dexter cows are mated with a Dexter bull a large proportion of the progeny are either still-born or deformed. As a result of constant disappointments, owners have gradually given up the attempt at breeding pedigree Dexters and, so far as Irish breeders are concerned, their whole attention is now directed to the development of the Kerry breed."

"Concurrently with the ban upon Dexters in Ireland, a boom was started in England." To supply the demand for Dexter cattle, the Irish breeders earnestly sought for methods by which Dexter-type calves could be produced and the monstrous calf avoided. The result of their experimentation has been that the Irish Dexter, the so-called "foundation stock" Dexter, is got not by a Dexter  $\times$  Dexter mating but by using a Dexter bull and Kerry cows. This mating has never yielded a monstrous calf; it has produced on the average equal numbers of good type Dexters and of "sneem" Kerries. The Kerry-type animal so produced is a diminutive Kerry; Sneem is the district of Kerry in which the foundation stock Dexter is raised for export to England, and "sneem" is used locally as a term of reproach, being applied to any undersized creature. The relative numbers of red and of black individuals varies considerably, for it is certain that many Kerries are heterozygous for their black coat colour character.

In England, the English Kerry and Dexter Cattle Society was founded in 1892, and published its first Herdbook in 1900. By the regulations laid down therein, "a cross between the Kerry and the Dexter is considered a half-breed and cannot be entered." Quickly, as Wilson says, "what was formerly known to Kerry men now became known to other breeders who bred Dexters, according to the rules of the Herdbook, that such a procedure invariably resulted in the production of a proportion of dead misshapen calves."

The abnormalities which these still-born calves exhibit are constant, and are so characteristic that the foetus is known as a "bull-dog" calf. The cranium is bulging, the nose markedly depressed, the lower jaw protruding, the upper lip is split, baring the teeth, while the swollen tongue, thrust far out, curls up over the nose. Owing to the disproportionate development of the buttocks, the tail seems to have its origin far up on the back; usually

there is a gaping deficiency of the abdominal wall through which the intestines pass to form a large umbilical hernia. The skin hangs loosely in folds ; there is abundant subcutaneous fat. The limbs are ridiculously short and the digits unusually separated.

The period of gestation in the Dexter is approximately 284 days. In the great majority of cases it can be foretold that a pregnancy is to terminate in the production of a "bull-dog" foetus, for in such cases the pregnant cow begins to increase in size very rapidly about the third or fourth month, and ultimately becomes very distended. The early obliteration of the hollow in the flank just in front of the hip is recognised as a sure sign of impending trouble. Then it is noticed that the cow is losing "water," which dribbles from the vulva, and that she is becoming less and less distended. After a time the loss of fluid ceases, but after a short interval the cow is as "big" as ever. Again there is the flow of fluid from the vulva and the decrease in size, and again the cycle is repeated. Following one of these discharges of fluid from the vulva the foetus is aborted. The fluid is described as being clear in the majority of cases ; in a few it has been turbid.

But, as Seligmann has previously recorded, it is not invariable for a Dexter with a considerable degree of excess of amniotic or allantoic fluid to give birth to a deformed calf. Moreover, the pregnancy which results in the abortion of a dead monstrous calf is not invariably associated with such excess. Extremely rarely, so the breeders say, the first indication of anything abnormal is a premature labour. In such cases the calf is not delivered naturally, it always must be removed by operative procedure and is dead when delivered.

The puerperium following the delivery of the "bull-dog" differs from that following the birth of a normal calf. The placenta comes away in small fragments or has to be extracted manually, instead of being thrown off complete in a half to four hours. In fact, herdsmen will state that there is no after-birth in the case of the "bull-dog." The lochia last longer than is usual, the blood-stained discharge persisting in certain cases even as long as a fortnight instead of the usual one to four days. The abortion of a foetus, other than a "bull-dog," is followed by an immediate cessation of mammary activity ; the abortion of a monstrous calf on the contrary does not interfere with this and the cow produces milk.

The normal Dexter calf is a small individual compared with calves of the same age but of larger breeds. No specimen of the normal Dexter foetus has been available for comparison, but an "off-type" (a Kerry-type) foetus



was obtained at the eighth month of pregnancy. The "bull-dog" foetus of the same age is very much smaller.

Age of "Bull-dog."	Weight.		Length.	Diameter Shoulders.	Length off Foreleg.	Length off Hindleg.
months.	lbs.	ozs.	cms.	cms.	cms.	cms.
7	12	15	28	44.1	5.1	5.7
7	10	3	30.5	42.6	7.3	7
7	11	11	30.2	39.9	6.1	5.7
4-5	6	11	17.8	30.2	6.1	6.4
3-4	3	8	20.5	17	3	6

An examination of many specimens has shown that there is never any suggestion of putrefaction or of mummification of the foetus. The abortion quickly follows the death of the calf. The death of the foetus is associated with severe foetal anasarca-foetal dropsy—in the case of the earlier abortions and of the majority of the later ones—and with profound dystocia in the cases which proceed until near term. In practically all cases in which there is hydramnios (or hydrallantois) foetal anasarca is present, and the foetus is a fluid-logged shapeless mass and the almost complete subcutaneous covering of the abdominal wall is devoid of skin over a circular area based upon the umbilical cord. In the case of the older foetus, death results from dystocia. The prolonged and difficult labour is made inevitable by the shape and consistency of the foetal head which cannot be accommodated by the maternal birth passages.

The "bull-dog" foetus is a creature which has a head of unusual shape and consistence. The size of the foetus and the shape of the head are to be regarded as significant features of the "bull-dog" calf.

In the herdbooks there are to be found many entries of "premature" or of "dead" in the column which shows the births during the year. It is not to be expected that such a record is critical. English Dexter breeders, anxious to have this problem solved, supplied the following absolutely trustworthy figures :—

Total births, 646; Normal calves, 530; "Bull-dog" calves, 116.  
i.e. 1 in 5.5 births.

At first sight there would seem to be neither rhyme nor reason in the occurrence of the monstrous calf. Some herds are singularly free from them, others yield so many that the breeder gets rid of his stock. A certain cow will produce a series of most excellent Dexter calves and then to the same sire will yield a typical monster. A cow to the service of several different bulls will produce a series of "bull-dogs," and then the next season produce a prize-winner. There is no Dexter out of a Dexter-by-Dexter mating that is not related more or less closely to a monstrous calf. The "bull-dog" appears in all herds in numbers that range from 5 to 30 per cent. of the total births. During the last two years the present writer has examined 27 cases.

Just as black is the more common colour of the Dexter and for the same reason, so is the coat colour of the "bull-dog" foetus more often black than red. Of the 27 cases examined, 20 have been black and the remaining seven have been red, as close an approximation to a 3 : 1 ratio as can be.

Of the 27 cases examined, 21 have been males and six have been females. For a long time none but a male was received and sex-linkage was suspected. But more recently six females have been examined ; at first these were regarded as possible cases of abnormal differentiation of the sex-organisation in a male, but the demonstration of definite ovarian tissue in the gonads has made it certain that the condition is not sex-linked. In the older foetuses the scrotum is well defined as is also the vulval cleft, but in the earlier specimens the sodden skin and the great rent in the abdominal wall make the identification of the sexual apparatus peculiarly difficult. Moreover, since the specimens cannot be examined until at least 18 to 24 hours after delivery the histological evidence is rather weakened. But the evidence is such as to show quite definitely that the "bull-dog" foetus may be either male or female, though, if the series of cases examined can be regarded as a representative sample, the majority of monstrous calves are male.

#### *IV.—The Pathology of the Monstrous Calf.*

*Foetal Anasarca* in the great majority of cases is present. This condition is not uncommon in cattle and is almost invariably associated with the death and abortion of the foetus at the sixth to seventh months. According to Williams, foetal anasarca has been recorded only in ruminants among the domesticated mammals. The cause is not known ; it has been ascribed by some writers to a congenital absence of the thoracic duct. In the case of the "bull-dog" it is invariably associated with hydrops amnii, and it is reasonable therefore in this case to suspect that both conditions are due to one and the same cause,

although in ruminants generally there is no doubt that the two conditions can occur quite independently.

*Hydramnios*.—The normal amount of fluid present in the amnion of the cow is about five to six litres, the amount in the allantois about six to fifteen litres. Any material excess is considered abnormal. The amniotic fluid is clear and the allantoic turbid in later pregnancy; the fluid evacuated in the case of the "bull-dog" is clear; the condition present in the case of the monstrous calf is probably hydramnios and not hydrallantois. When examined, no definite cell elements can be recognised in either stained or unstained preparations. The amount of fluid evacuated in one case was 160 litres approximately. Hydramnios is not uncommon in cattle and is the rule in such species crosses as *Bos americanus* × *Bos taurus* or *Bos americanus* × *Bos indicus*.

The exact cause of hydramnios is not known, nor can it be until the origin of the liquor amnii itself has been demonstrated. The prognosis in cases of severe hydramnios in cattle generally is very unfavourable both for cow and calf, for, as a result of the pronounced uterine dilatation and uterine paresis, the cervix is not dilated naturally and the abdominal muscles, losing tone for the same reason, do not aid in the expulsion of the foetus. Delivery is always by operative procedure, as is also the removal of the placenta. In the Dexter, however, the prognosis is entirely satisfactory as far as the cow is concerned; normal labour pains come on, the cervix is dilated, and the foetus is expelled without any artificial interference, unless it is an older foetus, for then the head cannot pass the pelvic brim and operative interference is imperative. The placenta must be removed manually in most cases. The difference in the prognosis in the case of the Dexter and other breeds rests on a difference in the general musculature. The Dexter is a peculiarly muscular individual and can withstand the stretching which hydramnios involves much better.

*Hydrocephalus*.—In one case, hydramnios and foetal anasarca were associated with pronounced hydrocephalus. Literally, there was no brain.

*The Skeleton*.—The shape of the skull of the monstrous calf is characteristic. Brachycephaly is marked, the cephalic index being about 100 per cent., and the skull being diminished in length and enlarged in width. The head is large with bulging forehead and parietal eminences; the nasal region is much depressed; the bones of the vault are thicker than is usual, and the most striking feature of all is the extreme shortness of the base. The supra-, ex- and basi-occipitals, the basi-, ali- and pre-sphenoids are fused into one mass of bone and no suture of any sort can be identified. The orbito-sphenoid is

distinctly delimited. In a normal Dexter foetus of seven months the basi-occipital measured 3.1 cm., and the basi-sphenoid 2.5 cm., giving a total antero-posterior length of 5.6 cm. when measured on the exterior of the skull, and one of 5 cm. when measured on the interior aspect. These combined bones in a "bull-dog" foetus of the same age measured 1.2 cm. and 1.3 cm. respectively. In the normal the foramen measured  $2.6 \times 2.8$  cm., in the "bull-dog" it was 1.8 cm. long and was waisted, measuring 0.9, 0.8, 0.9 cm. in width. No distinct condylar faces were discernible. Internally the ethmoid in the normal covered the orbito-sphenoid; in the "bull-dog" it did not. The vomer is extremely robust in the monstrous calf and has a very broad articulation laterally with the palatines. There is no hard palate and the nasal portion of the skull is completely exposed ventrally in consequence of the entire absence of any inward growth of the maxillæ and palatines. The posterior palatine processes of the pre-maxillæ are present, being fused with the anterior end of the vomer. The maxillæ are greatly fore-shortened as are also the nasals. The fore-shortening of the maxillæ is illustrated by the unusual prominence of the lachrymals and by the fact that while the general contour and proportions of the post-palatine processes of the maxillæ are normal, the pre-maxillæ, normal in design, practically come back to the level of the maxillo-palatine suture. Viewed ventrally, the pre-maxillæ lie much deeper than the inner margin of the maxillæ and palatines, the contour of these latter bones being such that there is no suggestion of any effort to make a palate. In this respect the skull is distinctly reptilian. The abnormalities of the skull are such as affect its posterior fossa, and save for various degrees of asymmetry and for the greater thickness of the vault, the remaining peculiarities are such as could follow from the abnormality in the ossification of the base.

The skull of the "bull-dog" foetus resembles that of the "bull-dog" in one respect only: both show fore-shortening of the facial region due to an arrest of development of the nasals and maxillæ.

In the case of the limbs of the "bull-dog" foetus the epiphyses of the long bones do not differ materially from those of the normal, but there is a profound difference in the length of the shaft. The shortening affects the proximal bones of the limb more than the distal. The digits are separated more widely than in the normal calf of the same age.

Sections of the epiphysial line of the long bones of the limbs show certain characteristic features. Normally, bone formation proceeds by (1) endochondral ossification at the junction of epiphysis and diaphysis, determining growth

in length, and (2) periosteal ossification determining growth in thickness. In endochondral ossification three zones can be distinguished in the area between the undifferentiated cartilage of the epiphysis to the diaphysis: (1) the zone of proliferating cartilage cells in which the multiplying cartilage cells lie in a clear matrix, (2) the zone of parallel columns of cartilage cells, in which the hyaline matrix is the seat of calcification, and (3) the line of ossification in which each column of zone extends as far as the bone marrow from which vascular loops extend to erode the cartilage columns in a regular manner. The septa separating the columns become eroded and denuded of their cells and become encrusted with calcareous salts, and on their surfaces osteoblasts are deposited by the vascular loops. These osteoblasts lay down successive layers of true bone whilst the calcareous material of zone 2 is removed by osteoclasts.

In the case of the monstrous calf zone 1 is unaltered, but on the surface of this there is a vascular fibrous area enclosing small islets of cartilage. There is no zone 2, the columnar arrangement of the cartilage cells being entirely absent and the cells, few in number, are scattered irregularly. The matrix is not hyaline but shows fibrillation, and the deposition of calcareous salts is irregular. Zone 3 is thin and irregular. Endochondral ossification occurs but the absorption of bone trabeculæ is defective so that reconstruction of the cancellous tissue fails. The trabeculæ are excessively thick, with comparatively small rounded areolæ between them. The condition is one of abnormal endochondral ossification, in that there has been defective absorption at the stage of secondary areolar formation. Periosteal ossification in these cases is normal and the abnormality is restricted to the epiphysial line where the cartilage cells are entirely passive, where there is no division, no column formation and no vacuolation and hypertrophy.

The vertebral column is also involved, in some cases being less than half the length of that of a normal foetus of the same age, and along with this abnormality there are others affecting the size and shape of the thorax and of the pelvis.

#### V.—*Diagnosis.*

In the search for a diagnosis it was necessary to review certain pathological conditions which affect the human subject. It was found that the lesions which characterise the "bull-dog" simulate very closely indeed some of the most typical features of achondroplasia as met with in the human.

The group name achondroplasia accommodates a considerable number of pathological conditions, which may or may not be different modes of

expression of a polymorphic disease. Such very different conditions have been described as micromelia, chondritis foetalis, osteogenesis imperfecta, pseudo-chondritis, cretinoid dysplasia, micromelia chondramalacica, osteoporosis, periosteal aplasia, and chondrodystrophia foetalis with its three varieties hyperplastica, hypoplastica and malacica. In this paper achondroplasia is referred to as a group name.

The human achondroplast presents the characteristic features of the condition. The trunk is shortened in some cases ; the limbs are short and thick and markedly curved with the convexity outwards ; the head is larger than usual with bulging forehead and parietal eminences ; the bridge of the nose depressed ; the skin thick and wrinkled. The foot is rotated inwards ; the vertebral column straight and the back flat though the buttocks and abdomen are prominent. The condition is reminiscent of the earlier foetal proportions, the limbs being short in relation to the trunk. Another persistent foetal character is the "main en trident (or bident)," the second and third metacarpals forming an angle of about 40 degrees with one another instead of the usual 32 degrees. In the newly formed foetal hand the metacarpals form an angle of  $\pm 90$  so that the digits are widely separated ; this divergence diminishes during the course of further development, but less in the case of the achondroplast than in the normal. Still another foetal condition is mirrored in the achondroplastic pelvis, which is smaller than the normal.

The great majority of achondroplasts are still-born, but a few survive to become robust muscular adults. The adult is of low stature and of great muscular development, has a normal sized trunk, a large head, and short limbs of the rhizomelic type—the proximal segments being more shortened than the distal—thick short hands and feet, and spread digits when extended. The shortness of the limbs is not due to any crookedness or bending of the bones as in rickets or osteomalacia, nor the result of multiple fractures as in osteogenesis imperfecta, nor to the congenital absence of any bone. All the customary bones are present but they are much shorter than is usual.

Achondroplasia in the human is a hereditary disease. An achondroplastic race cannot exist for the reason that an achondroplastic woman cannot come to a normal confinement, and in the absence of surgical interference both mother and child must perish. Cæsarian section is necessary in order to save both, and craniotomy must be carried out if the mother alone is to be saved. Micromelia and fore-shortening of the facial region, however, are familiar characteristics and they may be regarded as low grades of the condition of achondroplasia. In different cases the human achondroplast has

had an achondroplastic father, mother, brother(s), sister(s), grandfather, father and brothers and sons, an achondroplastic co-twin, a normal co-twin. An achondroplastic parent of either sex married to a normal mate may have normal children. Rischbeith gives a long series of such cases. It is of interest to note that Catherine de Medici, Natalie, sister of Peter the Great, and the Empress Ann of Russia tried without success to raise a race of these dwarfs by arranging their inter-marriages.

In the case of the human there would seem to be different grades of this condition ; the lowest grade being seen in the case of the adult of low stature whose arms and legs are short in relation to the trunk ; the highest grade being that seen in the still-born foetus which exhibits several if not all of the following characters :—shortened limbs and base of skull, depressed nose, harelip, abnormality of hard palate, narrowed foramen magnum, umbilical hernia, anasarca, hydramnios, prominent abdomen, thickened skin, abundant subcutaneous fat, apparent lordosis, brachycephaly, inturned feet, “main en trident,” shortened vertebral column. These are the very characters of the “bull-dog” calf.

The pathology of the condition has been described in detail by Emerson, Murk Jansen, Keith, Shattock and Sartorius, among others. It corresponds with the lesions found in the case of the “bull-dog” calf. The condition is not one of arrest of cartilage formation ; it is one of an arrest of bone formation in cartilage. The parts in the posterior fossa of the skull are arrested in their growth, there is considerable contraction of the foramen, great shortening of the basi-occipital and basi-sphenoid followed by a contraction of the nasopharyngeal space. The ossification of the pre-sphenoid is also arrested, and the distance of the pituitary fossa from the fronto-ethmoid junction is greatly reduced, with the result that the nasion is drawn inwards. The limbs show the condition of micromelia, the humerus and femur are affected more than the ulna and tibia, and the site of the lesion is limited to those lines where bone is replacing cartilage.

The conditions found in the case of the “bull-dog” calf are such as are found in the clinical and pathological entity known as achondroplasia. It would seem that the exhibition type Dexter itself is a low-grade achondroplast and that the “bull-dog” calf produced by the mating of two such individuals is a high-grade achondroplast, exhibiting the classical features of the condition in a most pronounced form.

*VI.—The Etiology of Achondroplasia.*

In the case of the human many and varied causes have been suggested : Bohn and Schwob as early as 1868 suspected a disturbance or an insufficiency of the placenta. Parrot (1876) considered that a congenital nutritive disturbance of the cartilage cells was responsible. Klebs (1889) suggested that a compression of the foetus by the umbilical vesicle was the cause. Von Franqué (1893) and Rindfleisch (1889) also suspected mechanical pressure. Dor (1893) suggested that an autointoxication was the cause. Poncet and Leriche considered that the achondroplasts constituted a distinct race, while Buck and Mayer (1900) held that the condition was a hereditary process and that the most severe cases were the last of a degenerate race. Porak and Durante (1905) inclined to the opinion that the condition was sclerosis of cartilage resulting from an autointoxication. Cestan and Regnault described the condition as a form of intrauterine rickets. Marie (1900) suspected abnormality of some gland of internal secretion, while Lugano and Devay agreed with Leblanc in regarding a malfunctioning of the thyroid as the cause. Collman described a case in which the thyroid was much enlarged, as did also Virchow and Neumann. Bowlby, on the other hand, records a case in which the thyroid was absent. Regnault, however, concludes that in the majority of cases this gland is normal. Vargas held that the thymus was responsible, while Parhon, Shunda and Zalplachta (1905) went further by suggesting that the condition was due to a combination of hypofunctioning of the pituitary, thyroid, and thymus, together with a hyperfunctioning of the sexual glands. Murk Jansen advanced an ingenious hypothesis to explain the conditions found in these cases. He argues that the responsible cause is a compression of the foetus by a too small amnion or by a hydramnios. Keith disagrees emphatically with Jansen's conclusions, pointing out that a comparison of such contrasted conditions as the fronto-cephaly of achondroplasia and opisthocephaly and simoproposia leads directly to the conclusion that such differences in end-results can be explained in terms of different action on the part of the agencies regulating growth. This growth-regulating mechanism is in all probability the endocrine system, and Keith argues that in the light of recent advances in endocrinology it is indeed probable that the stimulus which brings about the preparatory pre-ossification changes in bone formation is an internal secretion "such as we may expect to arise either in the pituitary, thyroid, suprarenal or genital glands, or by an interaction of secretions from all of these," and that the cause of achondroplasia is a malfunctioning of one or more of these glands of internal secretion.



The present study has not furnished a complete answer as to which, if any, of the glands of internal secretion is to be regarded as the responsible causal agent in the production of achondroplasia, granting, that is, that this condition in the "bull-dog" calf is achondroplasia. But this much is certain, there is profound abnormality in some of the ductless glands. If it is desired to investigate the cause of achondroplasia in the human, the most satisfactory way will be to maintain a herd of Dexters and to produce abortion of foetuses during the first three months of pregnancy.

*The Pituitary.*—In the case of the human achondroplast smallness of the pituitary has been commonly reported, and this fact has been deemed to be of some significance as it has been conclusively established that this gland is concerned in the normal growth processes. But no definite and constant histological abnormality of the pituitary has been found.

In the case of the "bull-dog" foetus the pituitary is definitely smaller and is more compressed than in the normal. Histologically, it presents the usual structure, save that in many cases there are areas of oxyphil cells in the *pars intermedia*. The cells of the anterior lobe appear to be more closely packed than is usual and the vascularity of the gland to be less than in the normal. It is not profitable to discuss the possible significance of such vague impressions as these. But advantage was taken of the melanophore test devised by Hogben and Winton in order to examine the functioning of the posterior lobe of the pituitary of the monstrous calf. These workers have shown that following an injection of a minute quantity of posterior lobe extract into a frog previously kept under those conditions which conduce to skin-pallor, there is a very characteristic and rapid darkening due to a marked expansion of the melanophores. In the case of the normal cattle foetus the pituitary is active, as estimated by this test, at the beginning of the third month. The pituitary is ground up in a mortar with sand and distilled water and the extract injected intra-abdominally. In the case of the "bull-dog" foetus, the pituitary of a six months' specimen gives a very doubtful reaction. A four months' pituitary gives a still more doubtful reaction. It is granted that this test is one for posterior lobe activity and that a malfunctioning of the pituitary which would constitute a possible cause of achondroplasia would be one involving the anterior lobe. Nevertheless it would appear not to be without significance that in the "bull-dog" foetus the posterior lobe is physiologically relatively inactive at the fourth and the sixth months; it is not unreasonable to think that the measure of the activity of the posterior lobe can be regarded as an indication of the functioning of the gland as a whole.

If this be so, then indeed there is reason to suspect that a malfunctioning of the pituitary during the earlier months of foetal life is responsible for the abnormalities in ossification and in growth. If, for example, the pituitary does not function properly at the time when the normal processes of ossification begin, and if, for the normal development of bone, the guiding stimulus of the pituitary is necessary, then a retardation of pituitary activity or an insufficiency of its secretion could lead to abnormality in bone formation, and the degree of imperfection in the end-results will vary with the degree of retardation of the pituitary functioning and with the difference in the time at which the different parts of the skeleton become ossified.

The suspicion that a malfunctioning of the pituitary may be involved in the causation of the conditions found in the monstrous calf is strengthened by the work of Krogh, who has shown that there is good reason to believe that posterior pituitary substance is concerned in the production and maintenance of capillary contractile tonus. Insufficiency results in capillary dilatation and oedema. Pituitary malfunctioning in this way can lead to anasarca, hydrocephalus and hydramnios. Moreover, it was found by Smith, and this has been confirmed by Hogben, that general oedema commonly followed injection of pituitary extract into larval Amphibians.

*The Thyroid.*—In the case of the "bull-dog" foetus Seligmann (1904) found that the thyroid was abnormal and concluded that the condition was one of foetal cretinism. In seven cases the thyroid was oedematous and purple; the isthmus absent or irregular in shape. Histologically, the gland consisted of masses of more or less cubical or spheroidal cells and the capillary network was extremely dense. Very few vesicles and sometimes only the faintest trace of a vesicular arrangement could be detected. There was complete or almost complete absence of colloid and the lumina of the vesicles were packed with cells. In 1911, Sheather described the thyroid as being normal in size, shape and histological structure, save that there was a slight excess of interstitial tissue in some parts of the gland. The vesicles were perfectly formed and filled with colloid. Crew and Glass (1922) described the thyroids of five foetuses and demonstrated that in these cases the thyroid did not show the histological features of a hypo-functioning but rather of a hyper-functioning gland. In the large series of cases which have now been examined the thyroid has varied considerably. In some, mostly the younger specimens, it has been unremarkable; in others it has been enlarged and the histological picture has been that of a thyroid from a case of hyperthyroidism, no colloid being present, the small irregular vesicles containing papillary ingrowths of epithelium and desquamated

material, and the section consisted of masses of solid cellular hyperplasia. In the older specimens the thyroid showed the typical signs of involution, the vesicles were enlarged, irregular and full of colloid, the epithelium low and the previously hyperplastic intervening tissue undergoing retrogression and transformation into fibrous tissue.

The sequence of events as suggested by the different histological appearances seen in different cases would seem to be, first, a developing thyroid, then a hyperplastic hyper-functioning thyroid, and finally an involuting gland with fibrous atrophy and progressive hypo-functioning. Such a scheme would accommodate the different descriptions which have been given by previous writers and would explain the diagnosis of foetal cretinism. This seriation of events is typical of cretinism, but it is also the typical course of events which follows removal of the anterior lobe of the pituitary in mammals, as has been shown by Cushing and more recently by Dott. The lesions found in the thyroid do not necessarily indicate that the condition is that of foetal cretinism. The mother of a monstrous calf is not goitrous herself. Shattock, in Seligmann's paper, suggests that the maternal thyroid is probably sufficient for the mother's needs only, but the experiments of Halstead and of Edmunds on the dog show that were this so the thyroid of the foetus would be greatly enlarged. The conditions found in the thyroid of the "bull-dog" foetus can be regarded as secondary to a malfunctioning of the pituitary.

Since Gudernatsch demonstrated the efficiency of thyroid feeding to accelerate Anuran metamorphosis, the value and specificity of this test, at least as an indicator of the iodine content of the gland, as demonstrated by Lenhart and Swingle, has been universally recognised. Using the axolotl, the larval form of the Mexican salamander, there is the opportunity of demonstrating thyroid activity in a most spectacular way, for a single meal of fresh gland suffices to induce metamorphosis. This is a critical test for thyroid iodine, the transformation does not occur in aquaria without this stimulus and it cannot be induced by the oral administration of inorganic iodine. This axolotl test has been shown to be a specific test for thyroid activity by Laufberger, Jensen, Kaufmann (L.), and Huxley and Hogben.

An axolotl weighing 64 grams was fed with 2 grams of fresh thyroid taken from a seven months' "bull-dog" foetus. Complete metamorphosis resulted, with shedding of the larval skin in the usual 12 to 14 days after the thyroid meal. A second axolotl of 23 grams was fed with 1 gram of thyroid from a four to five months' "bull-dog" foetus. Metamorphosis occurred with shedding of the larval skin at about the same time as in the previous instance. This

test cannot be applied successfully before the fourth month of intrauterine life in the case of the normal cattle foetus. From the fourth month onwards the thyroid is active as estimated by this test, before this it is not. It will be noted that according to the tests used, the pituitary is functional before the thyroid.

These observations give a clear demonstration that there is present physiologically active iodine in the thyroid of the monstrous calf of the Dexter at an early stage of foetal life. They do not support the contention that the condition is that of cretinism.

*The Adrenals.*—In no case have these been perfectly normal. In some there has been an undue amount of fibrous tissue in the cortex and in the medulla. In the majority there have been areas of cartilage and in many there are areas of cartilage bone with Haversian canals and areas of calcified cartilage with osteoblastic bone on its surface. The different stages in this bone formation would appear to be, hyperplasia of fibrous tissue, formation of hyaline cartilage in these areas, fibrillation of the cartilage, calcification in the matrix, absorption of the calcified cartilage by osteoclasts, and deposition of osteoblastic bone on the surface. The exact significance of this cartilage bone formation in abnormal situations has not been established, but since similar areas have been found in other organs in which there is plentiful connective tissue, it is suggested that the condition is a general one and possibly is secondary to a malfunctioning of the thyroid. In the cat, Blair Bell found areas of calcification in the adrenal after thyroidectomy.

No other abnormality of the endocrine system was encountered. It may be remarked, however, that in contradistinction to the general finding of sexual precocity in the living human achondroplast, the differentiation of the sex-organisation in the case of the monstrous calf of the Dexter is not so advanced as in a normal cattle foetus of the same age.

The tentative conclusions arrived at from this study are as follows:—Very possibly the condition results from a malfunctioning of the pituitary between the second and third month of intrauterine life. Under these conditions the proper control of cartilage bone formation is lacking. The thyroid undergoes hyperplasia, and this is followed by involution.

But it is not claimed that a definite answer has been given to the question as to the primary cause of the condition. It is felt, however, that the study of the monstrous calf of the Dexter has provided a strong argument in favour of using this material for a complete and thorough study of the conditions akin to achondroplasia. A small herd of Dexters would provide the finest

experimental material for a demonstration of the bearings of Genetics upon pathology and upon the science of animal breeding.

### VII.—*The Significance of the Monstrous Calf.*

The following points have to be considered :—

The Dexter had its origin in the mating of two distinct races of cattle and is peculiar for its bodily conformation characters, being in all probability a low-grade achondroplast. The mating of Dexter and Dexter results in the production of four classes of calves in such proportions as to suggest that the Dexter is a Mendelian di-hybrid in respect of its coat-colour and bodily conformation characters. A certain proportion of the calves produced by Dexter and Dexter matings are still-born and exhibit characters which constitute the condition of high-grade achondroplasia, the characters of their parents greatly exaggerated. Associated with these foetal characters there are usually present anasarca and hydramnios and in some cases hydrocephalus. The cause of death is either the cause of the hydramnios or the cause of the peculiarities in the skeleton which render normal delivery impossible. The cause, in all probability, is endocrinal in nature and is possibly a malfunctioning of the pituitary. The incidence of the condition is such as to suggest that the condition is genetical in its origin. There is but one way of producing a monstrous calf of this sort and that is to mate Dexter with Dexter. (Report has it that the same condition is found in the small Breton breed of cattle.)

These facts can be accommodated by the following scheme. The old-fashioned Kerry and the old-fashioned Devon furnished those factors which in combination yielded the original Dexter, a big-headed, stout-bodied, short-limbed individual. Or it may have been that the first Dexter was a mutation. It suffices to suppose that the original Dexter had the factorial constitution symbolised by the formula  $Bb (S\ 1_1\ 1_2) (s_1\ 1_2)$ . (The significance of the symbols  $1_1$  and  $1_2$  is indicated below.)

#### Dexter $\times$ Dexter.

56·25 per cent.	18·75 per cent.	18·75 per cent.	6·25 per cent.
Black Dexter-type.	Red Dexter-type.	Black Off-type.	Red Off-type.

Or 2 "achondroplasts" of the same grade as their parents and 1 non-achondroplast in every four on the average, while the remaining one would be a somewhat more pronounced case of "achondroplasia" than either of the parents. So far, the story is not one of pathology, for, considering what is known of the human subject, it is seen that a low-grade "achondroplast" is physiologically

efficient. In order to interpret the "bull-dog" calf in terms genetical, the following hypothesis is suggested.

During the formative period of the breed two independent mutations occurred. Each of these resulted in the appearance of a factor  $L_1$  and  $L_2$  respectively, the action of which intensified the action of the factors which resulted in the production of the low grade of the achondroplasia-like condition.  $L_1$  and  $L_2$  are modifying amplifying factors and their action is additive. Either alone produces a greater degree of the "achondroplasia" characterisation, and together they yield the highest grade which is seen in the non-viable "bull-dog" calf. The "lethal" constitution is  $SS+L_1+L_2$ . These mutations occurred in the early Dexter and not in the parental breeds, and would seem to be linked with the factor S.

Before the appearance of these mutant factors the gametes provided by the Dexter were  $B(S\ 1_1\ 1_2)$ ,  $B(s\ 1_1\ 1_2)$ ,  $b(S\ 1_1\ 1_2)$  and  $b(s\ 1_1\ 1_2)$ ; after the factors  $L_1$  and  $L_2$  had appeared independently, and in all probability in different individuals, the series of gametes would be as follows:—

A = $B(S\ 1_1\ 1_2)$ .	E = $b(S\ 1_1\ 1_2)$ .
B = $B(s\ 1_1\ 1_2)$ .	F = $b(s\ 1_1\ 1_2)$ .
C = $B(S\ L_1\ 1_2)$ .	G = $b(S\ L_1\ 1_2)$ .
D = $B(S\ 1_1\ L_2)$ .	H = $b(S\ 1_1\ L_2)$ .

Random matings in a population containing individuals of the following factorial constitutions

AA AB AC AD AE AF AG AH  
 BB BC BD BE BF BG BH  
 CC CD CE CF CG CH  
 DD DE DF DG DH  
 EE EF EG EH  
 FF FG FH  
 GG GH  
 HH

would yield a percentage of monstrous calves which would differ according to the relative numbers of the genotypes present in that population. The variety of the genotypes resulting is decided in great part by phenotypic selection. If it is assumed that red coat colour is not desired then the numbers of the EE EF EG EH FF FG FH GG GH and HH genotypes will be reduced very quickly. If it is assumed that the makers of the Dexter breed were aiming at as short-legged a beast as possible, that is, if it is assumed that

actually they were seeking the biologically unattainable, a high-grade "achondroplast" with the constitution represented by the formula  $SS+L_1+L_2$ , then the genotype BB would be discarded, for the breeder would choose his "best" individuals for further mating. The genotypes CD CH DG GH are the "bull-dog" calves and being still-born would not be available. So there would be left the following :—

AA	(S <sub>1</sub> l <sub>2</sub> ) (S <sub>1</sub> l <sub>2</sub> )	} Lowest grade of "achondroplasia" in the Dexter.
AB	(S <sub>1</sub> l <sub>2</sub> ) (s l <sub>1</sub> l <sub>2</sub> )	
AC	(S l <sub>1</sub> l <sub>2</sub> ) (S L <sub>1</sub> l <sub>2</sub> )	} Intermediate grade.
AD	(S l <sub>1</sub> l <sub>2</sub> ) (S l <sub>1</sub> L <sub>2</sub> )	
BC	(S L <sub>1</sub> l <sub>2</sub> ) (s l <sub>1</sub> l <sub>2</sub> )	
BD	(S l <sub>1</sub> L <sub>2</sub> ) (s l <sub>1</sub> l <sub>2</sub> )	
CC	(S L <sub>1</sub> l <sub>2</sub> ) (S L <sub>1</sub> l <sub>2</sub> )	} Exhibition Dexter, a higher grade but viable.
DD	(S l <sub>1</sub> L <sub>2</sub> ) (S l <sub>1</sub> L <sub>2</sub> )	

In random mating the incidence of the monstrous calf would be 12·5 per cent. of the total births. But if, as suggested above, the breeder persisted in selecting his animals for breeding from the  $SS+L_1$  and the  $SS+L_2$  individuals it is probable that only the genotypes AC AD CC and DD would be used, and in these circumstances the "bull-dog" foetus would appear much more commonly.

The proportion of "bull-dog" calves varies with the relative proportions of the different genotypes in the Dexter herd and with the amount of deliberate selection of individuals with certain characters which are held in esteem.

#### *VIII.—Suggestion as to Methods by which the Monstrous Calf might be Eradicated.*

According to the scheme that has been elaborated in this paper there are Dexters which, though of excellent characterisation from the point of view of the breeder, will not throw monstrous calves when mated with others of a similar genetic constitution. If the breeder altered his standards of excellence there would soon be no problem ; there are true-breeding races of domesticated mammals which present certain characters very closely akin to many of those which, in combination, constitute the condition of achondroplasia—the dachshund, Natas cattle and Yorkshire pigs are examples perhaps—and in such races it would seem that the amplifying lethal mutations have not occurred as they seem to have done in the case of the Dexter and of the Breton cattle. It is biologically possible to "fix" a degree of achondroplasia as a racial character. But the Dexter breeders are seeking to attain the biologically impossible—a highest grade "achondroplast" which is viable. Hydramnios and

dystocia render their efforts of no avail. They must be content to modify their standards somewhat ; an  $SS + L_1 + L_2$  individual cannot be produced, but an  $(S + L_1 + l_2) (S + L_1 + l_2)$  or an  $(S + l_1 + L_2) (S + l_1 + L_2)$  can, and these are very excellent Dexters. The methods suggested are as follows :—

*First Step.*—To get a herd in which all the individuals carry the factor  $S$  in the duplex condition.

Theoretically, the quickest way to do this is to mate several Dexter bulls and as many Dexter females as possible to Kerries and to retain only those which yield none but Dexter-type offspring. It must be remembered that the " foundation stock " Dexter is always Dexter by Kerry bred and so must always be heterozygous for its bodily conformation characters. If this is impracticable then the next best thing to do is to obtain Dexters which have never thrown " off-type " calves. There are such. In this way  $SS$  males and females will be secured.

*Second Step.*—To remove either the  $L_1$  or the  $L_2$  factor from the herd.

The males retained following the first step may have one of the following constitutions :—

$$A = (S L_1 l_2) (S L_1 l_2).$$

$$B = (S l_1 L_2) (S l_1 L_2).$$

$$C = (S l_1 l_2) (S L_1 l_2).$$

$$D = (S l_1 l_2) (S l_1 L_2).$$

$$E = (S l_1 l_2) (S l_1 l_2).$$

The females retained may be A, B, C, D or E.

Choose the best male and choose as young a one as possible. Mate him to as many females as possible. Discard all the females which, after repeated matings, produce a " bull-dog " calf. It is probable that either A or B will be the type chosen in the case of the sire. In the case of the females, types A and B are equally good Dexters, and C and D though not so good are by no means " off-types."

A mated with A will give no " bull-dog " calves.

A mated with B will give all " bull-dog " calves.

A mated with C will give no " bull-dog " calves.

A mated with D will give 50 per cent. " bull-dog " calves.

B mated with B will give no " bull-dog " calves.

B mated with C will give 50 per cent. " bull-dog " calves.

B mated with D will give no " bull-dog " calves.

C mated with C will give no " bull-dog " calves.

C mated with D will give 50 per cent. " bull-dog " calves.

D mated with D will give no " bull-dog " calves.



If the type E is used at all then it will not throw a monstrous calf in any mating, but it is assumed that the breeder wishes to get as low-set and bulky animal as possible.

Keep to one sire and discard every female that produces a "bull-dog": obtain a son of the sire out of a female that, after repeated matings with the sire, has not produced a monster and mate the son with the females which have not been discarded. Mate the sire to his daughters and discard all, and their mothers also, that produce a monstrous calf. In this way, if the sire is an A individual the B and the D females will ultimately be removed from the herd; if he is a B type animal then the A and the C type females will become removed. When this is accomplished, the monstrous calf will no longer appear.

*IX.—The Possible Bearing of the Case of the Monstrous Calf of the Dexter Upon the Species Question.*

The species-cross *Bos americanus*  $\times$  *Bos taurus* is complicated by the occurrence of hydramnios during the pregnancy, which results in the production of the F.1 generation, and by dystocia in the case of the F.1 male, which renders the birth of a male almost impossible. Moreover, if an F.1 male is born alive it is invariably sterile. The case resembles that of the Dexter in that hydramnios and dystocia are involved.

Certain breeders (Boyd, Goodnight) became impressed by the advantages which would follow the production of the "cattalo." Bison bulls were mated with Hereford and Aberdeen Angus heifers (the reciprocal cross could not be made, though the reason is not stated), and in every case the pregnancy was complicated by severe hydramnios, with the result that the majority of the females aborted. In fact, only about one in thirty produced a living calf and of these a male was a rarity, for the size and shape of the male hybrid were such that the mother died in labour in the great majority of cases. It was found that the size and shape of the head of the male and the length of his neural spines were such as could not be accommodated by the birth passages of the cow, and the calf or the cow or both died.

In this case analogous lethal factors are involved, complementary lethals resulting in the production of a degree or of a kind of development of the skeleton of the offspring which renders its natural birth impossible and in the production also of hydramnios which in a great number of instances ends in abortion. In this case, however, the factors concerned in the production of the conditions leading to dystocia are sex-linked and are not linked with

those which are concerned in the production of hydramnios. In the case of the Dexter monster the dissociation of hydramnios and dystocia has been recorded but very rarely, and has not been critically observed. In the great bulk of the cases the presence of a monster and of hydramnios in association is the rule so that the factors concerned are the same or they are so closely linked that their dissociation is extremely rare. The cattalo case differs also in that the surviving F.1 male is sterile.

The conditions met with in the bison  $\times$  cattle cross can be interpreted in terms of the factorial hypothesis as follows :—

A and A' are complementary autosomal factors, and together result in the production of hydramnios.

B is a dominant autosomal factor complementary to *d* a sex-linked recessive, and the combination Bd results in the production of certain skeletal characters which lead to dystocia.

C is a dominant autosomal factor complementary to *c*, a sex-linked recessive, and the combination Ce results in sterility.

The series *dc* is balanced by DE.

The bison male according to this scheme is AA BB CC (DEX)Y, the female AA BB CC (DEX) (DEX). The cattle male is A'A'bb cc (*de*X)Y, the female A'A' bb cc (*de*X) (*de*X). The F.1 male will be AA' Bb Cc (*de*X)Y, the female AA' Bb Cc (DEX) (*de*X). There will be hydramnios in all cases. Of the foetuses which continue to term there will be dystocia in the case of the male but in the female, since *de* is balanced by DE, parturition will be possible. Similarly, the female will be fertile, the male sterile. The fact that a few F.1 males are produced is to be explained by the variation in the maternal musculature, by differences in the size of the birth passages in cows of different ages and sizes, by differences in the foetal presentation, and by differences in the management of labour.

The females of F.1 were then back-crossed to the bison or to the bull, and this procedure was continued for several generations. It was found that, as time went on, the incidence of hydramnios became less and less and that the proportion of dead-born calves was steadily becoming reduced. Fertile males were obtained. These facts are easily accommodated by the scheme outlined above. It will be found that a backcross of an F.1 female to the bison sire will yield a generation in which the incidence of hydramnios is reduced by 50 per cent. as are also those of dystocia and of sterility in the case of the males. After a few years of breeding in this way several genotypes would exist and chance selection, guided to some extent by the breeder's art, would

surely in time completely remove hydramnios, dystocia and sterility. The peculiar results of this species-cross can be interpreted genetically, and looking at the problem broadly the case of the Dexter is very similar. Hydramnios characterises another species-cross—*Bos americanus* and *Bos indicus*—and one case of this was treated by Lewis, who performed hysterectomy in order to obtain a living hybrid.

The case of the monstrous calf of the Dexter suggests that in certain instances the factor of the geneticist may be endocrinal in nature, affecting the development of the tissues in such a way that conditions unfavourable to the foetus are produced. Dystocia may be the result of an abnormal size or proportion which cannot be accommodated by the maternal birth passages, and there is much circumstantial evidence which shows that proper growth is regulated through the mechanism of the endocrine system, and that abnormality of a component member of this system is followed by abnormality in the proportions of the individual. A "lethal" factor may be one which affects the proper and timeous functioning of a ductless gland.

A species, as recognised by the extreme systematist, is an association of individuals all of which exhibit a common morphological character complex; such species are commonly defined without any first-hand knowledge as to their behaviour as breeding units. It is perhaps due to this fact that most workers of the Mendelian school, with the notable exception of Bateson, have turned their attention from the historic problem of the origin of species to the more immediate question as to the origin of characters. Nevertheless, as Bateson has rightly pointed out, the fundamental discontinuity of species in the Linnean sense as breeding units has still to be interpreted in terms of the factorial hypothesis, which hitherto has shed much light on the discontinuity of animal structure but not upon the discontinuity of the breeding unit. Until further light has been shed on this issue the validity of the assumption upon which the evolution theory rests will not have found a satisfactory basis in experimental enquiry.

The species may be a gene-complex, as suggested by Morgan, and the morphological characters may be linked with physiological characters which really separate unit from unit. The case of the Dexter provides an opportunity for offering a suggestion as to the manner in which a discrete breeding unit may have its origin.

A mutant lethal factor may be such that in the simplex state its action is balanced by its normal allelomorph, but in the duplex state the combined action of the two results in the production of anatomical anomaly and physio-

logical derangement, of a kind that render the further development of the zygote impossible or profoundly abnormal. Such is the case of the homozygous yellow mouse. Or else, a mutant factor may be of such a nature that alone, either in the simplex or in the duplex condition, it produces no evident effects; but combined with a complementary factor of the same nature it results in a non-viable condition, and the mating of two individuals which carry such complementary lethal factors is rendered abortive.

A mutant appears in a stock; a lethal factor is present in the simplex state, and in the course of time there will make their appearance also individuals with this factor in the duplex state. The nature of the factor is such that alone it results in no appreciable effect. Synchronously, or at a different time and in a different race of the same stock, another mutation occurs and a factor appears whose action is complementary to that of the one referred to above, and in time individuals with this factor in the duplex state will be produced. Two distinct breeding units may thus arise within a common stock: each can successfully mate within its own group and with the parent stock, but the mating between the groups is rendered abortive. The expression of the action of such complementary lethal factors may take the form of incompatibility in the form of the copulatory apparatus, or in the physiological relationship between the male and female, or between the ovum and the sperm, of anatomical anomaly or of physiological derangement leading to an abnormal development of the zygote and its death, of hydramnios or of dystocia, or of sterility of the F<sub>1</sub> heterogametic sex. The two groups, however, have had their origin in a common germplasm and so, in the light of "return" and "regional" mutations, it is to be expected that parallel mutations will occur. Members of the two groups will exhibit characters which were borne by the common stock from which the groups arose, and characters which have resulted through mutation since the groups became distinct, some of these being the result of parallel mutation and some of mutation which has occurred in one group only. Such mutant morphological characters as are linked with the respective complementary lethals cannot be brought into genetic association and will become the distinguishing characters of the group.

#### *Summary.*

The Dexter is a breed of cattle of peculiar proportions which probably had its origin as the result of a cross between the indigenous Irish Kerry and the imported Red Devon.

The breed is remarkable in that a proportion of the calves are still-born

and present certain constant abnormalities which are closely akin to those constituting the condition of achondroplasia as met with in the human.

In this paper the incidence of these monstrous calves is interpreted in genetical terms, and a suggestion is made as to their eradication. Reasons are given for regarding a malfunctioning of the pituitary as the responsible causal agent in the production of the abnormality.

The possible bearing of the case of the monstrous calf of the Dexter upon the species problem is briefly discussed.

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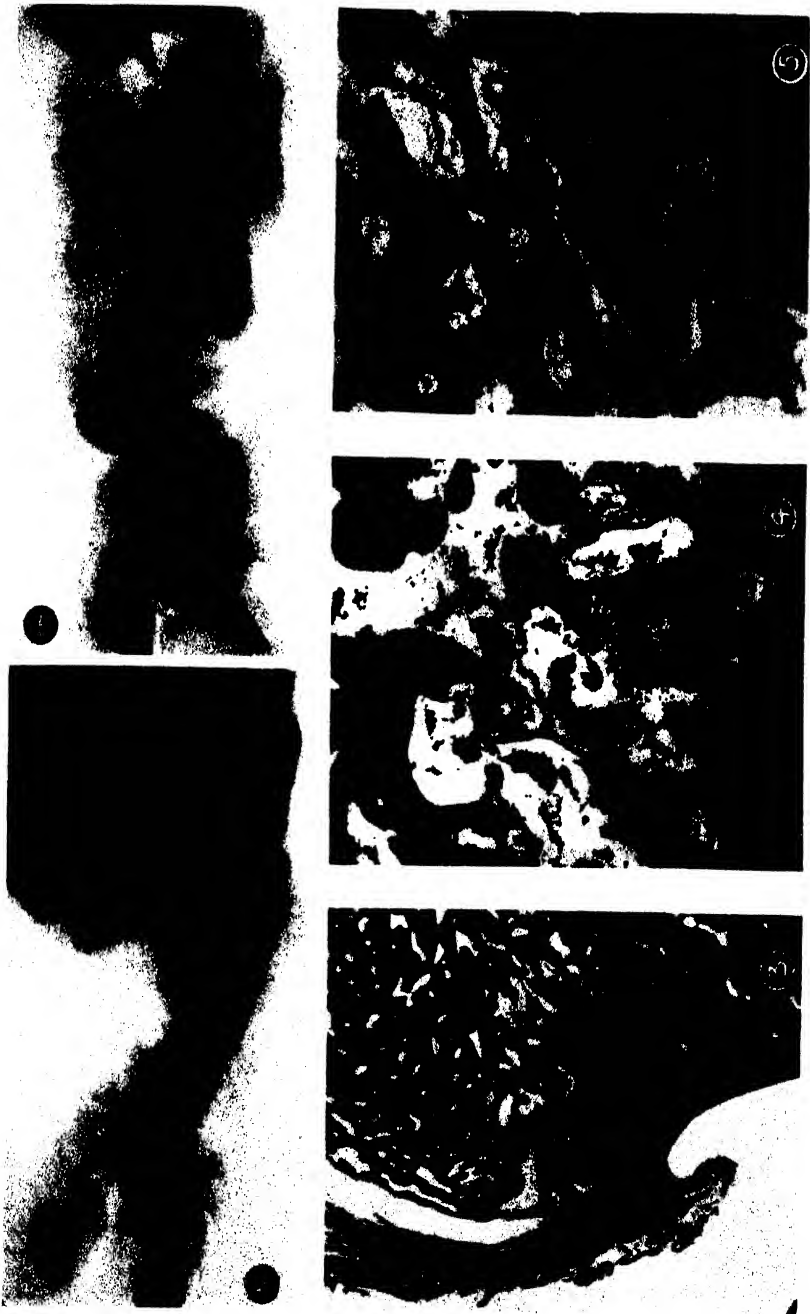


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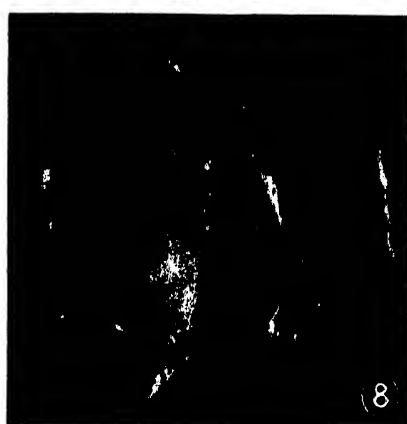
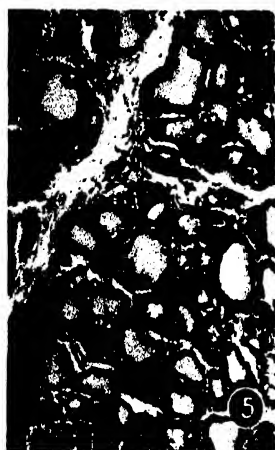
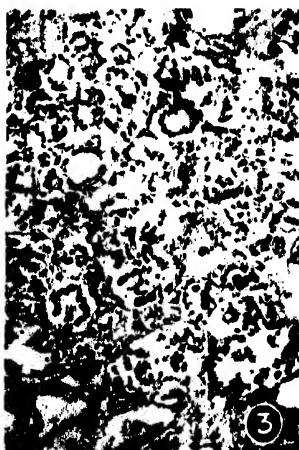


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DESCRIPTION OF PLATES.

PLATE 12.

- Fig. 1.—Six to seven months' "bull-dog" foetus, showing ventral hernia. Black ♂.  
Fig. 2.—Five months' foetus, showing wrinkling of the skin. Black ♂.  
Fig. 3.—Four to five months' foetus; hydrocephalus, anasarca, incomplete abdominal wall. Black ♂.  
Fig. 4.—Nearly full-time foetus. No hernia. Black ♀.

PLATE 13.

- Figs. 1 and 2.—The skull of a normal calf.  
Figs. 3 and 4.—The skull of a "bull-dog" calf of the same age.

PLATE 14.

- Fig. 1.—Radiograph of forelimb of "bull-dog" calf.  
Fig. 2.—Radiograph of hind-limb.  
Fig. 3.—Microphotograph of section through the end of a long bone, showing complete failure of reconstruction of cancellous bone.  $\times 22$ .  
Fig. 4.—Microphotograph of the epiphysial line, showing attempt at column formation.  $\times 110$ .  
Fig. 5.—Microphotograph of the area of calcified cartilage, showing failure of absorption of same and absence of reconstruction of cancellous tissue. Osteoblasts are present but fail to deposit osteoblastic bone. The epiphysis is normal. There is no sign of any cell proliferation and none of any attempt to form the serrated zone. The ossifying junction is occupied by a thick mass of osteoid tissue, which separates the cartilage from the diaphysis, and this area is non-vascular. The condition is one of abnormal endochondral ossification.  $\times 110$ .

PLATE 15.

- Fig. 1.—Thyroid of "bull-dog" calf, showing normal structure.  $\times 110$ .  
Figs. 2 and 3.—The same, showing hyperplasia.  $\times 110$ .  
Figs. 4, 5 and 6.—The same, showing involution.  $\times 100$ .  
Fig. 7.—The adrenal from a "bull-dog" foetus, showing the areas of cartilaginous bone-formation.  $\times 85$ .  
Fig. 8.—The pituitary of a "bull-dog" foetus, showing normal structure.  $\times 15$ .

*Studies in Intersexuality.—II. Sex-Reversal in the Fowl.*

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(Communicated by Prof. R. C. Punnett, F.R.S. Received 28th May, 1923.)

[PLATES 16, 17.]

§ 1. *Introduction.*

Many instances of "hermaphroditism" and of the more or less complete assumption of the secondary sexual characters of the male by the old female bird, wild and domesticated, have been recorded, but in the great majority of such cases no accurate knowledge of the previous history of the individual has been available and each has been considered as an isolated case. It was thought that a study of these conditions as met with in the domestic fowl would be of genetic interest. In order to obtain specimens and information the poultry keepers of the country were circularised. It was found that :—

- (1) Rare cases of "hermaphroditism" have been encountered. A bird at the age of 4–6 months, instead of developing the distinctive male or female characters, grows slowly into a creature which is neither a pullet nor a cockerel and its characters remain indefinitely intermediate.
- (2) Rare cases are known of an otherwise perfectly normal laying hen with cocky plumage. In one such case, known to the present writer, the plumage following successive moults has been cocky—henney—cocky.
- (3) The exhibition of the male type of sexual behaviour by a laying hen of perfectly normal external female characterisation is exceedingly common among the more highly fecund strains, and especially when no male bird is kept in the pen. This behaviour is corrected by removal into fresh company or by the introduction of a male bird.
- (4) The assumption of the male type of head furnishings—the larger comb (or the erect comb in certain breeds) and wattles—and the greater development of the spurs are by no means uncommon among 2–3-year-old hens of heavy laying strains. A hen, previously a heavy layer, ceases to lay, her head furnishings rapidly increase in size, the spurs grow; the plumage, bodily conformation and carriage remain henney; the bird makes effortful attempts to crow but exhibits no sexual behaviour and seeks solitude.

- (5) Such a bird as described above occasionally, following irregular moults, puts up more or less completely the type of plumage that characterises the male of her breed.
- (6) Very exceptionally such a bird as in (5) apparently undergoes a complete sex-transformation. She begins to exhibit the signs of a functional male activity: the crow becomes challenging and the disposition bellicose towards cocks, courteously masterful towards hens. The bird treads hens and fights cocks, and save for an indefinable difference in shape and carriage is a male, to be distinguished readily from a "real" cock only when compared side by side. Several poultry men of ripe experience have possessed such a bird, and several are in the hands of poultry keepers at the present time, but no case has been critically observed and recorded.

Unfortunately it remains the rule to kill and eat all such birds. Nevertheless it has been possible to secure some thirty specimens, and the examination of these has convinced the present writer that a rich field for study is laid bare. Two "hermaphrodite" hens have been obtained, and will be described later when a complete study of them has been made. Two specimens of group (2), normally behaved laying hens with cocky plumage, have been secured and mated up: before this could be done their tail sickles had to be cut, as it was found that otherwise the cock was inclined to regard them as males. A description of these birds is postponed until the genetic significance of the cocky feathering has been investigated. Class (3), the laying active birds of normal appearance exhibiting the male behaviour, can be dismissed, for examination of many cases has confirmed the conclusion of Pearl and Boring, that abnormal behaviour of this sort had no basis in any anatomical abnormality of the reproductive system or histological abnormality of the gonad. It may be, however, that such behaviour, indicative of an exceedingly active reproductive functioning, heralds incipient ovarian disease leading to the conditions met with in classes (4), (5) and (6), the graded condition in all being phases of one and the same process. A complete examination of all the other specimens has been made, and these have been divided into two main groups: (a) hens in which the ovary has been more or less completely destroyed by hæmorrhage, tumour or atrophy; and (b) hens in the gonad of which definite spermatatic tissue in some form was present. In this paper it is proposed to deal with the second group.

## § 2.

Certain cases have been recorded elsewhere which bear upon this study.

Tichomiroff (1887) described four cases of "hermaphroditism" in the fowl. All the fowls crow, but, except for the larger size of the comb and the presence of spurs of regular shape, were typical hens in appearance. The sexual behaviour was observed in two cases only and was found to be that of the male type. Male cloacal glands were present, but a normal somewhat under-developed oviduct and an abnormal ovary were found in one case. Microscopic sections of the ovaries showed that oocytes were absent in all, and that the sex gland was composed of dense fibrous tissue with a few cords of epithelial cells. The germinal epithelium was quite distinct and was in places invaginated into the stroma to form narrow sacs.

Brandt (1889) described three cases of the assumption of male characters by the domestic hen. The first was an old hen, partly cocky-feathered, in which there was a slightly developed oviduct and a small ovary containing numerous oocytes and branching cords of epithelial cells. The second was a hen with a very red comb and four long sickle feathers, who was crowing and did not lay. A normal oviduct was present, and there was found a left ovary containing normal and atretic oocytes and epithelial cords and tubules; the germinal epithelium covering the surface of the gonad was in places invaginating into the stroma. In addition there was a small right ovary containing follicles and epithelial cords. The third case was a henny-feathered fowl with rudimentary gonads and a small oviduct. No oocytes or follicles could be identified in either gonad, but epithelial cords and tubules were present in both.

Shattock and Seligman (1906-7) recorded two cases of "hermaphroditism" in the White Leghorn. In the first case the plumage was henny, the sexual behaviour indifferent, but the comb, wattles and spurs were as those of the male. Two sex glands, two oviducts and two vasa deferentia were present. The left oviduct was large, the right diminutive. The left gonad had the general appearance of a resting ovary, but on section it was found to contain but two oocytes and large numbers of tubules lined by a single layer of cells. The right gonad was much smaller and was found to be a testis with both active and inactive tubules. The second case was that of a bird which during the earlier part of its life had every appearance of a pullet but later assumed certain characters of the male. The head furnishings and the plumage were henny, but the spurs were unusually large and the bird played the part of the male in treading hens. On dissection a large oviduct and a lobulated sex-gland were found on the left side, and on section the gonad

was found to consist of three kinds of tissue : stroma, a close plexus of solid columns of epithelial cells, and numerous tubules lined by a single layer of epithelium.

Pearl and Curtis (1909) described a case of "incomplete hermaphroditism" in a Barred Plymouth Rock—a hen in every way, save that the head furnishings were as those of a cock. The bird occasionally made feeble attempts to crow but otherwise behaved like a female. A well developed oviduct and two gonads were present ; the left gonad was fairly large but contained no recognisable ovarian or spermatic tissue, being composed of indifferent cellular stroma. The right sex gland was small, irregular, and contained rods of cells bounded by a distinct basement membrane, whilst associated with it was an epididymis. No typical tubules were found.

Pearl and Boring (1918) described eight cases of "hermaphroditism" in the fowl. Five of these birds (Drentsch fowls, obtained from Herr Houwink, of Meppel Holland) had never laid an egg or exhibited any sort of sexual behaviour. None of them looked like entirely normal males or females ; the size of the comb, wattles and spurs varied so much in the different cases that it appeared to have no relation to the other sexual characteristics, such as carriage and bodily shape. The gonads of four of these birds were unpaired and showed no typical testicular tissue, although there were present cords of epithelial cells and small tubules, regarded by the authors as mesonephric in origin. In one exceptional case a small testis was found on the right side. The remaining three fowls described were more "hermaphroditic" in external characterisation than the above. "Atwood's hermaphrodite" had large spurs and male carriage, but the general shape of a female, a small comb and henny plumage. Its behaviour was bisexual. An infantile oviduct and two sex glands were present. The left gonad was an ovotestis with oocytes and seminiferous tubules in early spermatogenesis ; the right one was a small inactive testis. "No. 1329," a cross between Barred Rock ♂ and a Game ♀, was obviously a female when it first reached maturity, but gradually developed more and more male characters. It had the carriage, comb and spurs of a cock, but was henny-feathered and had no wattles, whilst its behaviour was entirely bisexual ; it never laid an egg. An oviduct and an unpaired gonad were present, and the latter proved to be an ovotestis, consisting mainly of functional seminiferous tubules. "No. 1616," a cross between Rhode Island Red ♂ and Barred Rock ♀, was at first a hen-feathered bird with a cock's head, but later the female characters became more prominent and the bird began to lay. After a time laying ceased, and the appearance of the bird became more male-like, the hackle

feathers were partly male, although the spurs and comb remained like those of the female. On dissection, an ovotestis with numerous oocytes and mature seminiferous tubules was found on the left and a small active testis on the right.

Hartmann and Hamilton (1922) gave an account of a hermaphrodite Rhode Island Red which at first had every appearance of a normal pullet, but later assumed certain male characters and exhibited male sexual behaviour. It remained henny-feathered, laid on one occasion, and twice adopted chickens, yet it developed the head furnishings and crow of the male and would fight with other cocks. Dissection revealed an oviduct, two vasa deferentia, and two gonads, of which the left was an ovotestis with large oocytes and active seminiferous tubules, whilst the right one was a functional testis.

### § 3. *Description of Cases.*

It is now proposed to describe in detail eight new cases. All these birds were kept under observation for eighteen months and then killed by decapitation. I am greatly indebted to Miss H. B. Fell, who has carried out the histological part of this study for me. It is intended that she shall give a more complete description of the histology of these cases in a separate paper.

No. 1.—In February, 1921, a Buff Orpington was given to this Department; in the preceding January its owner decided that there was far too much crowing for a city in her pen of Buff Orpingtons—consequently she had the cock killed. Shortly after this it was noticed that a great deal of crowing was coming either from this pen or from somewhere near by, and at length it was found that one of the hens was responsible for the noise. This hen (Fig. 1) was  $3\frac{1}{2}$  years old, a pure-bred Buff Orpington, a good layer and a mother of chickens. On closer examination it was seen that the head of this bird was somewhat male-like, for her comb and wattles were rather larger than those of the typical hen. In fact, her owner concluded that some one had taken out a hen and replaced her by a cockerel.

On her arrival here the bird exhibited the classical signs of early ovarian disease. She had ceased to lay in the autumn of 1920 and had moulted. Her comb was a single,  $2\frac{1}{2}$  cms. at its highest point; she had spurs 3 mms. long on the left leg, 2 mms. long on the right; the wattles were somewhat larger than those of the typical Buff Orpington hen; the plumage was entirely henny. She crowed weakly as one practising and her sexual behaviour was indifferent: she did not behave as a cock towards hens or as a hen towards a male.

In April, 1921, the vascular tissue of the head had become markedly tur-

gescent, so that the eye appeared to be deep-set amid flaming-red congested flesh, the comb, wattles and spurs had progressively increased in size, and the bird had begun to moult irregularly. The feathers of the neck, saddle-hackle, and tail, as they were renewed, were seen to be cocky in structure. At this time the bird was obviously not well and suffered from an intractable diarrhoea. She was losing weight and sought solitude. Still the moult continued and by October she had become entirely cocky-feathered, though she could never retain the tail sickles. The spurs were now about 1 cm. in length, the left one being slightly longer than the right, and the legs had assumed the red tinge which characterises the male of the Buff Orpington. She was carefully nursed through the winter, and it became evident that the more urgently dangerous symptoms were overcome, for the bird exhibited a certain well-being. By February she was crowing lustily and with a challenging note, was readily attracted by hens which would squat on its approach, and the sexual act would be performed. The bird fought with any and every male in the yard and was gently courteous to the hens. In fact, only by one accustomed to poultry or when placing it alongside a "real" cock, could it be told that this bird was different from a typical male. Its stance differed from that of a cock; the bird was shorter on its legs, which formed a different angle with the body.

On February 3rd, 1922, the bird was placed with a hen, a virginal Buff Orpington, in a pen far removed from all other birds. This hen was laying; the eggs which she laid during the fortnight previous to her mating were incubated and found to be infertile. Every egg she laid after the mating was incubated. Her mate performed the sexual act daily: fluid passed into the cloaca of the hen was withdrawn and examined; on April 23rd a few living spermatozoa were identified in the fluid. On June 16th the hen became broody and nine of her own eggs laid during the preceding eighteen days were placed under her. On July 7th two chickens were hatched: the other eggs were clear.

Towards the end of June the cocky-feathered Buff Orpington became again ill. No longer did it act as a robust male, but became sexually indifferent, had ceased to crow, and on palpation a large tumour cephalad to and discrete from the gizzard could be felt. Severe diarrhoea again set in and the bird began to lose weight rapidly. It was removed and carefully nursed in the hope of carrying out further mating in the spring of 1923; but the bird fell into an opened drain and was drowned on December 29th, 1922.

At the time of death the comb at its highest point measured 4 cms., the right spur was 4 cms., the left 5 cms. long, the plumage entirely cocky but



very scanty. When the abdominal cavity was exposed it was seen that the liver was of great size and thickly studded with rounded yellow areas of tumour growth; it weighed 340 grammes. The proventriculus, gizzard and intestine were carefully dissected out; all of those were the seat of plentiful tumour growth, taking the form of rounded yellow masses based upon the peritoneal coat. The tumour growth had also invaded the mucous coat of the large intestine. Further examination showed that the condition was one of abdominal tuberculosis.

Lying in the situation of the ovary was a rounded mass  $7 \times 4$  cms. in size, with its purple surface marked with raised areas of yellow. Dissected out from the dorsal body-wall this mass weighed 52.5 grammes. Incorporated in the dorsal aspect of this mass there was a structure exactly resembling a testis, whilst another similar in appearance was situated in the equivalent position on the other side of the body. The two testes-like bodies measured  $3\frac{1}{2} \times 2$  cms. and had even outlines and surfaces, although the one on the left was flattened and bare of peritoneum where it had been related to the tumour mass. The adrenals were larger and more prominent than is usual. There was nothing remarkable about thyroid and pituitary. On the left a thin straight oviduct could be identified, having a diameter of 3 mms. in its widest part near the cloaca; paired vasa deferentia were clearly discernible. The tumour, testes, oviduct, and vas were removed for histological examination.

On sectioning, the structure of the gonads confirmed the conclusion that they were functional testes in a phase of reduced activity. The seminiferous tubules were precisely similar to those of the testis of a normal cock; they consisted of a well-defined basement membrane lined by seminal epithelium showing every stage of spermatogenesis. They differed from the tubules of a very active testis in that they were smaller in size and showed fewer mitotic figures; ripe spermatozoa were usually present in the lumen but not in large numbers. The intertubular tissue, as in the case of the normal cock, occurred in small quantities, owing to the very small interstices between the tubules; it consisted of connective tissue only: no "luteal" cells were present. Both testes were invested by a well developed fibrous tunica albuginea. Only one Wolffian body was sectioned; it resembled an epididymis rather than a parovarium, as a large distinct lumen was always seen in the ducts, which were lined by columnar ciliated epithelium and usually contained groups of ripe spermatozoa. The tumour proved to be the ovary, almost completely destroyed by tubercular disease.

The bird just described had been up to the age of  $3\frac{1}{2}$  years an unremarkable

hen; she had laid many eggs and raised many of her own offspring. Her history was known, since her owner kept but few fowls. In the autumn of 1920 she began to suffer from ovarian disease, which became noticeable in January, 1921. The disease was tuberculosis of the ovary, which progressively removed the ovarian tissue and so produced the effects of pathological ovariectomy. But it would seem that this tumour growth in its effects so altered the general metabolism of the individual that the conditions favourable to the differentiation and growth of spermatic tissue were created. New sex cords developed from the germinal epithelium and spermatic tissue was differentiated both in the left gonad and also in the incompletely atrophied right. The bird became anatomically equipped to function as a male, for with the development of the testes the Wolffian ducts were apparently stimulated to form functional vasa deferentia and the cloacal apparatus of the male was developed. Synchronously with the replacement of ovarian tissue by spermatic the oviduct atrophied. The bird functioned as a male and became the father of two chickens.

If this is indeed a case of complete sex-reversal in the fowl, in which a zygotic female as a result of the disturbance of metabolism by tumour growth had become a somatic male, and if the fowl has the *Abraxas* type of sex-constitution, then the sex-ratio of the offspring of this bird and a normal hen should be 50 : 100, so :—

P <sub>1</sub>	XY		XY	
Gametes	X	Y	X	Y
F <sub>1</sub>	XX	XY	XY	YY (an infertile egg or a dead zygote)
	♂	♀	♀	
	1	2		

Of the two offspring of the mating, both typical Buff Orpingtons, one is a male and the other a female. These have been inter-bred and their progeny are typical Buff Orpington chickens.

No. 2.—The bird was a Rhode Island Red, hatched in February, 1921. The owner, when sending the bird, wrote: “. . . the sex of which I cannot tell. It is to all intents a pullet and looks almost as much like a cockerel.” The head of the bird was as that of a pullet, there was no saddle hackle, the neck hackle contained some feathers distinctly intermediate in form, the tail was completely henny. Spurs were present on both legs, the one on the right measuring 1 cm., the one on the left 0.3 cm. Sexual behaviour indifferent. Did not crow. On dissection, the internal genitalia were found to consist: on the right, of a testis measuring 1.2 × 0.5 cms.; on the left, of an ovo-

testis,  $4 \times 2$  cms., with the spermatic portion nearer the mid-line of the body. Two oviducts were present, the left one 33.4 cms. in length  $\times$  1 cm. in diameter; the right one  $20.2 \times 1$  cm. Two vasa deferentia could be identified.

*Histological Examination: Left Gonad.*—The general structure of the organ was that of an ovary consisting of a cortex and fibrous vascular medullary cords, but the bulk of the cortex was composed of cords of epithelial cells similar to those described by Pearl and Boring in the gonads of certain abnormal hens. The testicular component was represented by immature and fully developed tubules occurring throughout the organ. The ovarian tissue consisted entirely of cystic follicles of various sizes, no normal oocytes being present. The cysts, which were occupied by a thin amorphous material and usually a few erythrocytes, projected above the surface in clusters, though smaller follicles were found among the epithelial cords. "Luteal" cells were very abundant and occurred throughout the gonad as large conspicuous masses of cells with vacuolated cytoplasm and very definite cell walls. The mature tubules of the spermatic tissue occurred chiefly in large groups situated in the more central parts. In none was spermatogenesis very active, and spermatozoa, when present, were few in number; spermatids were almost always found, but the majority of germ cells were spermatocytes in different phases of synapsis; mitoses occurred in a few instances but were usually totally absent. In the more peripheral areas many of the tubules were in degeneration.

The histological features of this gonad made it clear that typical testicular tissue arises, in what has been an ordinary cystic ovary, from a new set of sex cords proliferated from the germinal peritoneum covering the surface of the ovary.

*Right Gonad.*—The small right gonad was composed of three types of tissue: (1) large functional seminiferous tubules; (2) atrophic tubules; and (3) undifferentiated sex cords and large groups of "luteal" cells. The mature tubules contributed about one-third of the organ and were typical in structure. A few fully formed spermatozoa were present, but, as in the left gonad, spermatogenesis apparently had not been very active and mitotic figures were uncommon. As in the normal functioning testis, the tubules were closely packed together and the small interstices were filled with loose connective tissue. The atrophic tubules formed large groups lying between the fully developed tubules and the sex-cord region; their structure was similar to that of the resting tubules of the left gonad, but they were more degenerate. The syncytial cytoplasm was scanty and showed the fibrillar appearance in a marked degree, while mitotic

figures could not be found. One of these tubules appeared to have been regenerating and contained spermatozoa in the pachytene stage. The lumina rarely showed a central cavity. The intertubular tissue was dense and interstitial groups of "luteal" cells were common. The sex-cord region consisted entirely of the undifferentiated cords, as in the case of the left gonad. The invaded tissue appeared to be chiefly plain muscle which, however, was only recognisable near the periphery owing to the necrotic condition of the tissue in the centre. Enormous quantities of deeply staining amorphous material was found among the cords and was especially abundant in the central parts. Many of the cords were degenerating and showed great nuclear hypertrophy and thin granular cytoplasm. Large groups of "luteal" cells were present among the cords. The entire organ was enveloped by much muscular and fibrous tissue, which in the region of the functional tubules formed a regular albuginea with numerous blood vessels.

Attached to one end of the gonad was a group of small ducts, lined by rather shallow columnar ciliated epithelium, which evidently represented the Wolffian body. The ducts were embedded in a stroma of connective tissue. Lying nearest the gonad were a number of anastomosing vessels lined by cubical epithelium, probably representing a rete.

No. 3.—A Rhode Island Red, which was regarded by its owner as a henny-feathered cock; it crowed but very exceptionally and only in answer to another; its behaviour was peculiar—it played the part of a male towards other birds but was never observed to tread. The feathering generally was henny, but many of the neck-hackle feathers were intermediate in structure, though much more henny than cocky. The comb measured at its highest point 4 cms., the wattles were 5 cms. long; the left spur was 2.5 cms. long, the right one was represented by a mere button measuring 3 mms.

On dissection, the internal genitalia were found to consist of a small testis on the right side, and on the left of an ovo-testis irregular in outline, with pediculated ovarian portions containing convoluted atretic follicles. On the right side there was a vas deferens and on the left an oviduct 10 cms. in length and about 3 mms. in diameter near its termination.

*Histology: Left gonad.*—The bulk of the gonad was composed of functional testicular tissue and the ovarian elements were confined to the extreme periphery and the interlobular clefts. The ovarian tissue was represented by (a) numerous oocytes of various sizes; (b) cystic follicles containing a thin amorphous material; (c) numerous large discharged or atretic follicles, easily recognisable with the naked eye, occupied by vacuolated cells, many of which contained

yellow pigment. In the intertubular clefts the ovarian tissue was distinct from the testis portion with which it was connected by fibrous vascular cords. Groups of atrophic tubules, similar in structure to the resting tubules in the ovo-testis of No. 2, were, however, occasionally seen in the stroma. Elsewhere the ovarian and testicular constituents occurred together as a solid block of tissue. The oocytes were typical and contained a large vesicular nucleus, with chromosomes in the late diplotene (chiasma) stage. Beneath the nucleus, Da Fano preparations showed the large aggregate of mitochondria and crescentic Golgi rods characteristic of the oocytes of the fowl. All the largest and many of the smaller oocytes were in degeneration. The membrana granulosa had thinned out to a mere line, and the follicle was finally reduced to one of the cysts to which reference has been made. In some cases the oocytes were undergoing the usual form of atresia, *i.e.* resorption by the proliferating granulosa cells. The so-called "luteal" cells were plentiful, both as the usual small islets in the thecæ externæ of the follicles and also in larger masses unassociated with a follicle. Islets could also be seen among the mature testis tubules. Hæmatopoietic foci showing granulocytoblasts in different stages of formation occurred in various regions.

The spermatic tissue was best developed in the central portion of the gonad, where it was composed of large tubules in active spermatogenesis. These formed a much greater proportion of the organ than was the case in the ovo-testis of No. 2, in which the fibro-cellular cords of the hilus, though reduced in number and size, were still distinct and not, as in the present instance, completely obliterated by mature testicular tissue. The tubules, which were separated by comparatively wide spaces occupied by loose areolar tissue, showed every phase of sperm formation, and spermatozoa were almost invariably present. Towards the periphery of the gonad the tubules became steadily less differentiated in character. At first, though still in active spermatogenesis, they were seen to be of smaller cross-section, more closely packed together, and embedded in a denser fibrous stroma. Next, spermatozoa and spermatids were no longer present and the seminal epithelium consisted chiefly of spermatogonia and primary spermatocytes, most of which were in the contracting pachytene phase. Finally, for some distance beneath the surface the tissue consisted of cords of cells among which the oocytes were scattered. The cords, as usual, were composed of oblong or polyhedral cells containing a large spherical nucleus, occupied by a few small granules of chromatin and a plasmosome. Mitoses were numerous, and synaptic figures, though most frequent in the more central portions of the cords, occurred throughout their length. Da Fano

preparations showed that the mitochondria were of the ordinary filamentous type and that the Golgi apparatus was identical in structure with that of the germinal epithelium of the tubules, i.e. it consisted of a split horseshoe-shaped rod enclosing the archoplasm. Even at the extreme periphery of the ovotestis functional tubules were occasionally found, and sometimes occurred as groups of large tubules situated in a protuberance from the surface of the gonad. The fibrous capsule enclosing the protuberance usually contained heaps of the so-called "luteal" pigment, and it seems probable that this represented an old discharged or atretic follicle. In those parts of the gonad which were occupied by the sex cords the peritoneal epithelium was seen to consist of greatly enlarged cells, which formed a layer of deep columnar epithelium, sharply limited from the subjacent tissue by a basement membrane. Mitotic figures were common, and here and there typical synaptic figures were seen. At intervals the basement membrane was ruptured and the peritoneal cells were in continuity with one of the sex cords. No active regions of proliferation were found, as in No. 2. Darkly staining colloid was present in some regions in the cortex of the ovotestis, but occurred in relatively small quantities.

*Right gonad*, as was anticipated, proved to be a functional testis with every tubule in active spermatogenesis. It presented no abnormal features.

No. 4.—A White Leghorn hen, 3½ years old, had, to the surprise of its owner, within a short time assumed the head furnishings of the male, had begun to crow, and exhibited a bellicose disposition. In its general attitude it behaved as a male towards cocks and towards hens, but did not tread. The spurs grew in length, the right one being at the time of the bird's death 1·8 cms., the left 1·7 cms. long; the comb was 5·8 cms. at its highest point, but it never became entirely erect. The plumage remained completely henny, save that three of the neck-hackle feathers were rather intermediate. On dissection there were found on the left a yellowish smooth-surfaced sex-gland of an irregular shape and measuring 1·5 × 5 cms., a thin oviduct 5 mms. in diameter, and a vas deferens.

*Histology*.—The gonad was found to be a cystic ovary with a heavy secondary growth of testicular tissue. It was divided into two parts, one of which consisted of ovarian stroma, cysts, atrophic tubules, and fibrous vascular cords, and the other of cysts and normal immature tubules. The two parts were separated by old blood clots, groups of fat cells, and dense masses of fibrous tissue permeated by syncytial plain muscle fibres. The part composed of recognisable ovarian tissue contained cysts which were mostly simple, as in the ovotestes of Nos. 2 and 3, but were sometimes lined by columnar epithelium

and were papilliferous. They contained the usual mucoid matter. No oocytes were found, but the remains of old discharged or atretic follicles were common. These projected above the surface as ovoid structures and were formed of large groups of vacuolated cells, embedded in dense fibrous tissue. The cortical region was thin, except where large lobules of atrophic tubules occurred. These tubules were almost identical in structure with the resting tubules described in the left gonad of No. 2, but were more degenerate. Mitotic figures occurred but were rare, and no synaptic figures were seen. Occasionally a normal immature tubule, with a central lumen and regularly disposed epithelium, was found. In many cases the spermatogenic tissue appeared to have been proliferated into a discharged or atretic follicle, and the intertubular spaces were occupied by groups of vacuolated pigment containing cells resembling those described in the old follicles. Ordinary "luteal" cells were also present and were readily distinguishable from the pigment-containing cells by their larger and clearer nucleus, smaller vacuoles and faintly staining cytoplasm. Many sex cords in process of transformation into "luteal" cells were seen. Elsewhere the tubules seemed to have been formed in the stroma, and in other areas in which there was less evidence of degeneration the intertubular tissue consisted only of loose fibrous strands, while the whole area was enclosed by fibrous walls, suggesting that the tubules had been formed in a large cyst. The tubules were everywhere closely wedged together and often distorted in shape accordingly. The peritoneum was nowhere thickened and proliferation of sex cords seemed to have ceased.

The other portion of the gonad consisted of several large and numerous small cysts and typical young seminiferous tubules. The large cysts contained a small quantity of mucoid substance, were about 2 mms. in diameter and were lined by ciliated columnar epithelium with no papillæ. Some of the small cysts which were probably less distended showed the papilliferous structures and were lined by thicker epithelium. The seminiferous tubules were enclosed in a fibrous capsule, which almost undoubtedly represented the remains of a large multilocular cyst. Comparison with the testis of a normal 3 months chick showed an almost identical appearance in the two cases. The shape and size of tubules, their degree of convolution, the size of the lumen and the cytological character of the epithelium were the same in both, and the only appreciable difference was the presence of a greater number of definite spermatogonia in the normal testis. The tubules were bounded by a well-marked basement membrane and lined by a layer of high columnar epithelium composed of narrow cells with indistinct cell boundaries. A small but distinct lumen

was present. Occasionally one or more larger cells occurred lying more centrally: these had a large round nucleus containing more chromatin granules than the other nuclei and were probably definitive spermatogonia. Mitotic figures were fairly common. Some of the tubules contained a central mass of cell debris, which was usually giving rise to a concretionary body—a phenomenon which was also seen in the normal chick testis. One or two of the tubules were seen to branch. The intertubular tissue, unlike that of the normal chick testis, was extremely sparse, although the intervening spaces were often fairly wide; it consisted of a few fine fibro-cellular strands and numerous small blood vessels. In some parts a fine coagulum was seen, which was probably part of the original cyst contents.

No. 5.—Like the previous, this bird was a White Leghorn hen, henny-feathered but with the head-furnishings of a cock. The comb was 5.7 cms. at its highest point and completely erect; the right spur measured 1.8 cms., the left 1.7 cms. The sexual behaviour was indifferent: the bird did not lay, crow, or tread. On dissection, a small atrophic ovary was found on the left side; it was yellow in colour and most of its surface was flat. A small oviduct, 5 mms. in diameter, was present.

*Histology.*—The ovary was found to be completely atrophied and even degenerate oocytes were not present. In places, a few small irregular cavities containing coagulum and a few erythrocytes were present, which perhaps represented the remains of cystic follicles. The peripheral part of the gonad, where the oocytes would normally have been situated, was occupied by atrophic tubules such as those described in the preceding member of the series. They were of irregular shape, closely packed together, and contained loose degenerate cytoplasm and a few shrunken or hypertrophied nuclei. In some cases the basement membrane had been broken through and the germinal epithelium was being invaded by connective tissue. Material fixed by the Flemming and Mann-Kopsch methods showed that large fat globules were often present. No mitotic or synaptic figures were seen. The germinal peritoneum showed in most regions the characteristic histological changes which immediately precede the proliferation of sex cords. It was much thickened, the nuclei contained polarised loops and the basement membrane was being penetrated. In some areas the basement membrane could not be made out, and the peritoneal cells seemed to be more or less continuous with the underlying tissue. The most central part of the ovarian cortex was principally occupied by large, dense aggregates of polyhedral sex-cord cells, amongst which lay much deeply stained colloid substance. In some areas the cells were arranged in closely compressed



columns, but usually they had no definite arrangement. Large numbers of cells were in places undergoing transformation into "luteal" tissue.

No. 6.—This bird was likewise a White Leghorn, 3 years old, concerning which the owner wrote: "For some time she has been treading other hens exactly as would a cock-bird." The bird was henny-feathered and had the head furnishings of the male; the spurs were but 0.5 cm. in length. During all the time it was under observation it did not crow or tread other hens; neither did it lay. On dissection, a "resting" ovary and oviduct were found.

*Histology.*—The gonad was found to be a cystic ovary, in which the stroma was to a great extent replaced by undifferentiated sex cords and very young tubules. Numerous irregularly shaped cysts of the simple type described in Nos. 2 and 3 were present, but neither oocytes nor old discharged follicles could be found. The sex cords and tubules were very closely packed together, the latter far more numerous than the former, but there was no sharp line of demarcation between the two as they graded into one another. Mitotic figures were fairly numerous but no synapsis occurred. In some areas many tubules were degenerating through a rather peculiar process of liquefaction. Groups of "luteal" cells in every stage of formation from sex cords occurred throughout the gonad, and in places large areas of cords and small tubules were found, all of which were undergoing this process. Occasionally a few larger atrophic tubules were seen, with the usual scanty fibrillar cytoplasm and scattered nuclei. Small groups of normal immature tubules, resembling those described in No. 4, were sometimes present at the periphery and appeared to occupy a cystic follicle. They were lined by a columnar epithelium, and a small central cavity was always present. Examination of the peritoneal epithelium did not reveal any of those changes which characterise an incipient sex-cord formation, and it would appear, therefore, that the production of cords from this source had ceased. New spermatid tissue probably arises from multiplication of the cells of the cords previously proliferated. The Wolffian body consisted of a number of ducts lined by ciliated columnar epithelium. The lumina of the ducts were usually occupied by coagulated fluid. A rete, lined by rather indefinite cubical cells, was in close relationship with the ducts.

No. 7.—Like the three preceding numbers of the series, the bird was a White Leghorn, 3 years old. Its plumage was henny, the head furnishings large and vascular, the spurs mere buttons. The bird did not lay and did not crow or tread. On dissection, there were found a small yellow atrophic ovary measuring  $2 \times 0.5$  cms., and a small, straight, thin oviduct.

*Histology.*—The ovary was found to consist of a rather thick cortical region

and a few very fibrous central cords. Numerous shrunken cysts projected above the surface, representing the remains of follicles, but oocytes were absent. The cortical portion was composed partly of connective tissue stroma and partly of sex cords which varied considerably in size. In places they were large enough to be regarded as small tubules, such as those described in No. 6, and consisted of a basement membrane lined by polyhedral epithelial cells, which completely filled the lumen. Such tubules were often seen to be in connection with the thickened peritoneal epithelium, from which they were evidently being proliferated. A few were in colloid degeneration and others gave rise to conspicuous groups of "luteal" cells. Large areas were occupied by smaller cords, usually not more than one cell in thickness, which towards the centre lost their characteristic structure and formed a close syncytium of irregularly disposed cells. Many of these were either in degeneration or in process of transformation into rather compressed "luteal" cells. Other regions of the cortex were mainly composed of typical ovarian stroma, in which were seen large numbers of small degenerating "luteal" cells and a few necrotic sex cords.

No. 8.—The bird was a Light Sussex, 3 years old, concerning which its owner wrote in the spring of 1921: "This bird laid for us up to the time of coming into moult last autumn (1920), and since then has assumed a male appearance, its appendages have developed, but it does not crow; it has ceased entirely to lay." The bird did not crow or lay all the time it was under observation. Its plumage remained henny but the head furnishings were male in type. The spurs were small, the right being 1.7 cms., the left 1.5 cms. long. In 1922 it went broody and raised chickens.

*Histology.*—The structure of the gonad was found to be that of an atrophic ovary with numerous lobules of sex cords and young seminiferous tubules. The cortex, except when occupied by spermatid tissue, was very thin and was composed of the usual form of ovarian stroma, with here and there a lymph nodule. An occasional cystic follicle was met with, and more rarely the remains of a small oocyte, which had passed through the ordinary process of involution. The medullary cords presented the same appearance as those of the normal ovary. In some areas the peritoneal epithelium was thickened, the nuclei contained distinct polarised loops, and incipient sex cords were beginning to grow inwards into the stroma. The majority of the sex cords were rather large and might almost have been described as young tubules. Their histological character was precisely similar to that of the cords in the ovary of No. 5. They usually occurred as large spherical or ovoid masses, which projected from the

surface of the ovary, and which on macroscopic examination had been regarded as discharged or atretic follicles; it seemed probable that the cords had been proliferated into such structures. They may also occur in the stroma, although this was uncommon. In places, groups of larger atrophic tubules were found; one or more normal immature tubules were also often found in such groups. "Luteal" cells occurred in great numbers, and their origin from sex cords was excellently demonstrated in Mann-Kopsch preparations, in which the "luteal" cells were very deeply impregnated and stood out black against the surrounding tissue. As is the case in the embryo, the first stage in their development appears to be an increase in the number of mitochondria, followed by the formation of numerous small clear vacuoles, of which the chemical nature of the contents is as yet unknown. The nucleus becomes reduced in size and is stained and impregnated more deeply than in the neighbouring cells.

#### § 4. *Discussion.*

The histological study of the gonads in the foregoing series of abnormal fowls demonstrates beyond doubt that the birds were originally hens, the ovaries of which atrophied at some period of life and were then invaded by peritoneal tissue. This tissue in some birds consummated development by giving rise to mature seminiferous tubules, as in the case of Nos. 1, 2 and 3, and in others produced undifferentiated epithelial cords, which either continued to grow indefinitely, thus developing into tubules of an embryonic or immature type, as in the gonads of Nos. 4-8 of this series, or formed a malignant tumour as in other birds examined. Traces of the proliferation of sex cords from the germinal peritoneum are met with in most of the ovaries, but the process is particularly well shown in the left gonad of No. 2, which also demonstrates the homology between the undifferentiated cords, atrophic, and mature tubules, which otherwise it would have been difficult to establish beyond criticism.

The first bird in the series is perhaps the most clear-cut instance of sex-transformation in vertebrate animals yet recorded, and the cases display a consistent seriation illustrating the conversion of an actively functioning female into an actively functioning male. It is shown to be a fact that a fowl, which previously had been equipped with the sex-organisation of the female and had functioned as such, may undergo such a transformation as to come to possess the sex-organisation of the male, and actually to function as a male. Ovarian tissue is replaced by spermatid, and the type of differentiation of the rest of the sex-equipment pursued under the direction of the functional ovary gives place to that type which is pursued under the direction of the functional testis. It

is necessary to bring these facts into line with the established principle of the zygotic determination of sex in the fowl and with the more general problem of the over-riding of the sex-chromosome mechanism.

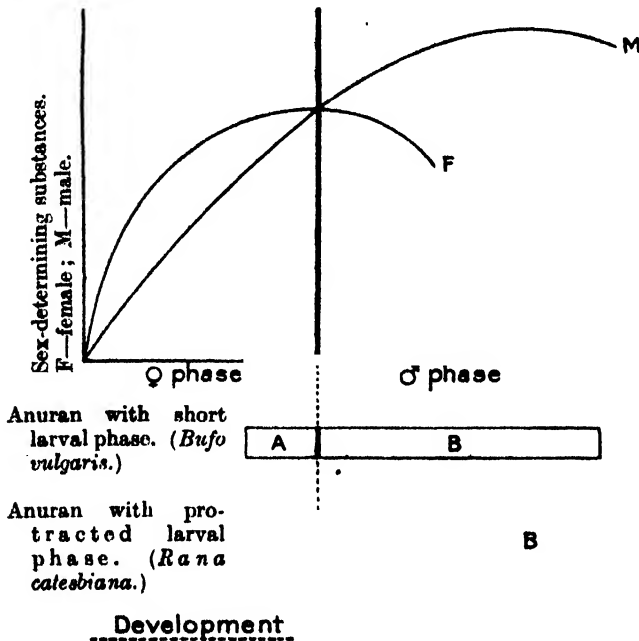
The actual sex-chromosome constitution of the fowl has not been finally demonstrated, but the indisputable evidence of sex-linked inheritance affords strong reason for holding that sex in the fowl is determined at the time of fusion of the gametes by a sex-chromosome mechanism of the *Abraxas* type, the sex-chromosome constitution of the male being symbolised as XX, that of the female as XY. If this is so, then, as in the case of *Drosophila*, it might be expected that any agency disturbing the balance between the two sets of sex-determining factors—i.e. those borne upon the X-chromosome, and those borne on the autosomes, in the cytoplasm, or possibly on the Y-chromosome—would produce a greater effect in the XY individual, in this case the hen. The fact that one of these fowls, kept under closest observation, had during the first three years of its life the appearance, behaviour and functional powers of a hen and then later assumed the attributes of a cock, makes it clear that the type of sex-organisation and of the reproductive functioning of the individual are not irrevocably decided by the sex-chromosome constitution. It is certain that an XY individual—a “determined female”—can produce sperms just as efficiently as it can produce ova. The crude definition that a female is an individual which elaborates ova and a male one that produces spermatozoa requires profound modification: the transformation of the sex-organisation of an individual, a determined male or a determined female, into that ordinarily possessed by an individual which has the alternative sex-chromosome constitution is an established fact. Before actual breeding work had been carried out with such sex-transformed individuals, many authorities were inclined to interpret the histological features of the gonads in these cases as instances of degeneration. Swingle, for example, in his vigorous criticism of Witschi's recent work on the frog, appears to suggest that any ovum-like bodies found in the testis of a “determined male” are to be looked upon as instances of “oviform hypertrophy or degeneration” of spermatogenic elements; and presumably, therefore, any spermatozoon-like bodies found in the ovary of a “determined female” as the result of a “testiform degeneration” of the ovary. But when such products of “degeneration” play their part as functional gametes, as is the case in Goldschmidt's latest experiments with *Lymantria* or in the first bird of this series, must it be assumed that a method of genesis then occurs which is fundamentally distinct from ordinary bisexual reproduction? It has been tacitly assumed that an oocyte or a spermatocyte possesses its peculiar cyto-

logical features in virtue of *its own* nuclear sex-chromosome constitution. But such a dogma is becoming more and more untenable, since it fails to account for a number of well-authenticated facts and is unsupported by critical evidence. Actually, an oocyte is a germ-cell in the meiotic phase characterised by certain features, of which the principal are the intercalation of a diffuse post-diplotene stage, with the active enlargement and proliferation of the plasmosome, and growth of the cytoplasm correlative to it. These features are undoubtedly not entirely restricted to oocytes, for in the spermatocytes of such forms as *Saccocirrus* and of some *Hemiptera* a very transient diffuse stage is seen; whilst in the protestis of *Bufo* and in the functional male gonad of some *Chilopods*, all the characteristic features of the oocytes are to be found.

A more reasonable view would seem to be that the cytological features of the oocytes or of the spermatocytes depend, just as does the character of the somatic structures of the rest of the sex-equipment of the individual (*cf.* the differentiation of the sex-mosaics of *Lymantria*), on the balance of conflicting physiological factors determined by the sex-chromosome constitution of the body as a whole, or, in the case of birds and mammals, locally through the action of the interstitial cells of the gonad. On this assumption Goldschmidt's conception of a timing mechanism puts the discussion, as to whether ovum-like bodies in a testis are oocytes or not, on an entirely new footing, and shows that such questions as the homology of Bidder's organ to an ovary or the transformation of a characteristically female definitive gonad to one of the male type does not in the least conflict with the view that genetical factors play an important rôle in sex-differentiation. If it is agreed that the essential difference between the male and the female lies in the timing mechanism which decides whether, whilst a given organ is developing, the male or the female determining reactions are predominant, if it is agreed that at some point in the development preceding or following the stage of differentiation the female determining reactions are predominant in a "determined" female and *vice versa*, then such questions as the above are resolved into a mere verbal quibble, and the efficiency of environic agencies to co-operate with the genetical factors offers no difficulty. The provisional hypothesis outlined by Goldschmidt to account for his *Lymantria* intersexes then brings into one coherent scheme undoubted sex-transformation as seen in *Crepidula*, *Sacculina*, and *Bonellia*, the occurrence of oviform cells in the protestis of *Anura* (or among *Myriopods* as steps in normal spermatogenesis), and the cases of sex-reversal met with in birds.

Text-fig. 1 shows how the significance of Bidder's organ, for example, can readily be interpreted on Goldschmidt's hypothesis. A represents the pro-

gonad, B the definite testis. It will be remembered that the development of the progonad is *actually later* in the forms with a protracted larval phase.



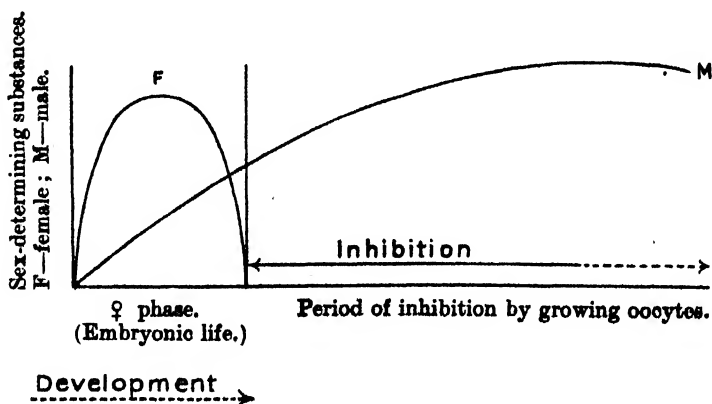
TEXT-FIG. 1.

During the earlier part of the development of the individual the female determining reactions are predominant, the female determining substances are effectively in excess, and any organ that is developed during this period and is responsive to the action of their stimulus will pursue its development and differentiation under their direction; later, in the case of the determined male, the male-determining substances become effectively in excess, and all differentiation of the structures of the sex-organisation will thenceforward be according to the male plan. Thus it will happen that in the case of the *Anuran* with a short larval phase, the pro-gonad will present the characteristic features of Bidder's organ, whereas in the case of the *Anuran* with a protracted larval life, in which the progonad is developed later, there will be no Bidder's organ and no ovum-like bodies in the testis.

In the case of the fowl it has been shown that there are successive invasions of the organ by sex-cords derived from the peritoneum. The histological appearances suggest that so long as growing oocytes are present these invading sex cords do not develop further into functional germinal tissue, but possibly

become transformed into "luteal" cells. But in the absence of growing oocytes these cords are apparently converted regularly into seminiferous tubules. It would seem that the physiological conditions which in the embryo at the time of the differentiation of the sex-organisation induce the primitive germ-cells to assume the characters of oocytes—and it will be remembered that these are laid down before birth in the fowl—no longer obtain in the mature bird, so that if what may legitimately be regarded as some inhibiting influence of the functional ovary upon the invading sex-cords be removed, as is the case in ovarian atrophy and disease, or, to put the matter differently, if by ovarian disease the conditions favourable for the continued development of the sex cords are created, the germ cells inevitably take on the characteristics of spermatogonia, spermatocytes, and spermatozoa. This conception, which appears to arise naturally from the histological data, is fully consonant with the fundamental postulate of Goldschmidt's hypothesis: that the essential difference between a determined male and a determined female is not a qualitative difference in the physiological processes involved in sex-differentiation, but is a difference in the time-relation of these processes in their operation upon the course of development.

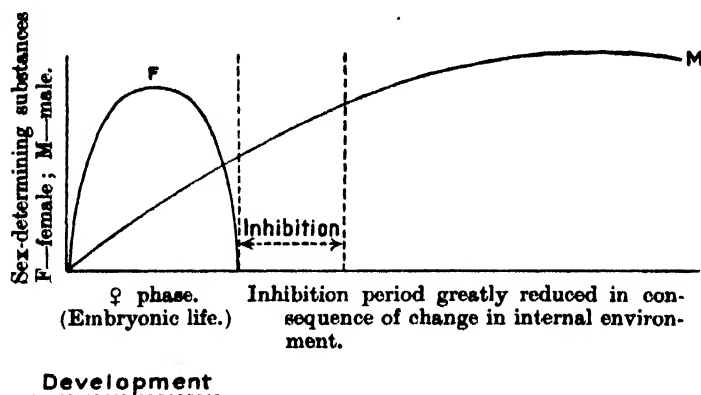
The phenomenon of sex-reversal in the case of the female of the domestic fowl interpreted in terms of Goldschmidt's hypothesis can be illustrated so. (Text-fig. 2.)



TEXT-FIG. 2.

During embryonic life the female-determining substances are effectively in excess and the differentiation of the gonad and the rest of the sex-equipment proceeds under the influence of the female-determining reactions: the oocytes are laid down. Ordinarily during the succeeding years of the individual's life

the growth of the oocytes precludes the operation of the male-determining reactions which are increasing in efficiency. But should the conditions be



TEXT-FIG. 3.

unfavourable for their growth, or should the conditions favourable for the continued development of the sex cords arise, as a result of the physiological exhaustion consequent upon excessive egg-laying or from hæmorrhage or tumour-growth, then, in the absence of the inhibitory influence of the growing oocytes, the male-determining reactions become effective, spermatic tissue is differentiated and the characters of the individual become those of the male. (Text-fig. 3.) It can be expected that almost any hen of a highly fecund strain will sooner or later develop some degree of the male characterisation.

### Summary.

1. A number of fowls are described which display a consistent series illustrating the conversion of an actively functioning hen into an actively functioning cock.

2. The fact that such sex-transformation can and does occur is brought into line with the established principles of zygotic determination of sex in the fowl, and with the more general problem of the over-riding of the sex-chromosome mechanism by the application of Goldschmidt's conception of a timing mechanism in sex-differentiation.

3. It is shown that in the hen there are successive invasions of the ovary by sex cords derived from the peritoneum, and that the assumption of the male characters by the hen is prevented by the presence of the growing oocytes. In the absence of the inhibiting influence of the growing oocytes the male-deter-



mining reactions become effective, spermatic tissue is differentiated from the invading sex cords, and as a result of this the characters of the individual become those of the male.

I am greatly indebted to my friend and colleague, Dr. L. T. Hogben, for his ever-ready help and much constructive criticism in the course of this investigation.

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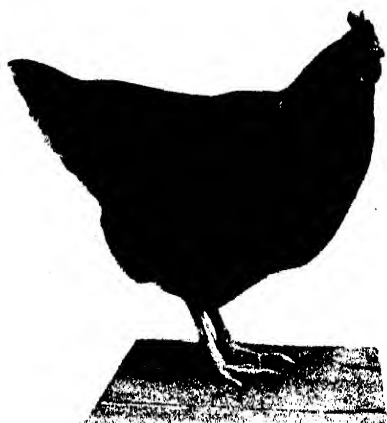
#### DESCRIPTION OF PLATE FIGURES.

##### PLATE 16.

- Fig. 1.—Case No. 1, p. 260.  
Fig. 1a.—Case No. 1, p. 260.  
Fig. 2.—Case No. 2, p. 263.

##### PLATE 17.

- Fig. 3.—Case No. 3, p. 265.  
Fig. 4.—Case No. 7, p. 270.





## *The Influence of Mechanical Conditions of the Circulation on the Electrocardiogram.*

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[PLATES 18 AND 19.]

### COLD-BLOODED HEART.

*Previous Work.*—As a preliminary to the investigation of the effect of work on the mammalian heart it was advisable to carry out experiments on the cold-blooded heart in order to determine the relations of the electrical deflections to the amount of work performed. Special attention was paid to the duration of the electrical response and the constancy of the heart rate. The isolated ventricle of the tortoise (*Testudo græca*) was used.

In the frog's heart Straub (1) measured the effect on the electrogram (direct leads) of increasing the filling and initial tension. Under these conditions he noted that the R' and T' waves were diminished in size. Seeman (2), working on the same animal but using a slightly different method, found that with increased venous pressure R' and T' diminished in the same ratio; little or no change in the electrogram was noticed on raising the pressure against which the heart contracted. If the heart was allowed to beat under isometric conditions, R' and T' diminished as the venous pressure was raised, until a certain level was reached; at this point the amplitude of the deflections showed no further alterations. Seeman concluded that these changes were not brought about by the augmented work of the heart muscle but by the adjustment of tension differences during contraction. Working on the frog's heart, Dale and Mines (3) demonstrated the balance between the frequency of beat, the duration of electrical response, auriculo-ventricular and intraventricular conduction; they also showed the effects of nervous influences on these factors.

*Methods.*—The isolated ventricle was set up in the apparatus employed by Frank (4) and by Kozawa (5) for measuring the isometric and isotonic response of the heart to varying conditions of inflow, tension and filling, and arterial resistance. The present procedure was substantially the same as Kozawa described with additions for taking electrograms during the heart contractions. Göthlin's modification of Ringer's solution was employed as a perfusion fluid and the whole apparatus placed in a thermostat. Zinc sulphate, kaolin, and sodium chloride non-polarisable electrodes were placed on the base and apex of the heart and led off to an Einthoven's string galvanometer, from which

standardised curves were obtained. Monophasic deflections were obtained by cauterising the muscle beneath the apical electrode; it was necessary to recauterise if the experiment lasted longer than ten to fifteen minutes, as at the end of this time a diphasic response commenced to appear. Electrograms were taken of the isometric contraction of the ventricle with varying degrees of filling. The tension developed during contraction was recorded by means of a Hürthle manometer, the movements of the lever being registered simultaneously with the electrical deflections on the photographic plate. The fall of the plate was timed to take place at a definite interval after the heart had been filled with fresh fluid, since the length of time the fluid remained in the heart affected the value of the components measured.

*Results.*—The effect on the isometric contraction response and electrogram of increasing the filling of the heart will be seen in Table I. The heart rate is nearly constant. The period of rising tension has been measured from the beginning of the rise of the manometer lever to the summit of the curve, and the duration of mechanical response (D.M.R.) from the commencement of the rise to the point where the lever has descended half-way to the original base line. This measurement is more accurate than an estimation of the duration of the whole mechanical response, since the curve falls off very gradually at the end.

The duration of electrical response (D.E.R.) was taken from the upstroke to the end of the final deflection.

It is evident from the Table that when the tortoise ventricle is beating isometrically, increased cardiac filling prolongs the period of rising tension, the D.M.R. and the D.E.R.; in addition, the maximum tension developed is increased and the height of electrical response (H.E.R.) decreased.

With the exception of the two experiments given in Table I, the heart rate became more frequent as the filling was augmented, indeed it was a matter of difficulty to maintain the rate constant. The cause of this greater frequency was the production of premature beats arising in different parts of the ventricle. In a few instances the rate went up and the form of the electrogram was unchanged, presumably the rate of stimulus initiation or the excitability of the muscle at the same focus was increased. In confirmation of Ludwig and Luchsinger (6) the same phenomenon has been observed to take place in the isolated ventricle of the frog on raising the pressure of the perfusion fluid. The importance of carrying out the experiments at a constant heart rate is evident from the work of Mines. In the other hearts in which the rate accelerated, the several values measured moved in the same direction as the constant heart-rate experiments. The acceleration was insufficient to mask wholly the effects of raising the cardiac filling.

TABLE 1.—EFFECT OF FILLING ON THE ISOMETRIC CONTRACTION RESPONSE OF THE TORTOISE VENTRICLE.

	Filling c.c.	Mm. Hg.		Period of Rising Tension. secs.	Duration of Relaxation secs.	D.M.R. secs.	V-V secs.	D.E.R. secs.	H.E.R.	Temp C°
		Max. Tension.	I.T.							
9.12.21. Heart Wt. 1.8 gm. Monophasic Variation	0.5	10.0	1.2	1.78	0.36	2.14	5.60	2.48	16.0	25.0
	1.0	15.5	0.5	1.89	0.52	2.41	5.48	3.43	16.0	24.5
	1.9	19.0	0.9	1.91	0.78	2.69	5.72	4.01	3.0	24.9
16.12.21. Heart Wt. 0.7 gm. Diphasic Variation	0.10	6.0	0	1.42	0.35	1.87	4.90	2.03	19.0	23.0
	0.20	20.6	0.4	1.63	0.59	2.22	4.78	1.95	10.0	23.0
	0.40	23.0	0.7	1.80	0.76	2.56	4.85	3.64?	6.0	23.0
	0.60	27.0	1.3	?	?	2.62	4.87	4.87	3.0	23.0

I.T. = Initial Tension.

D.M.R. = Duration of Mechanical Response.

V-V = Length of Cycle.

D.E.R. = Duration of Electrical Response.

H.E.R. = Height of Electrical Response.

## MAMMALIAN HEART.

*Introduction.*—From considerations of the work of Lewis (7) and others in relation to the path taken by the excitation wave, it did not seem improbable that the reaction of the heart to altered mechanical conditions might determine the duration and decline of the excitation in the ventricular muscle, although conduction in the Purkinje system might be unaffected.

Einthoven (8), Müller and Nicolai (9), investigating the changes in the E.C.G. occurring during and after exercise, found that the T-wave was increased in amplitude. Rehfisch (10) in a series of clinical cases, observed that as the blood-pressure and size of the heart increased, the amplitude of R and T deflections became larger.

Lewis and Cotton (11) showed that the changes in the human E.C.G. after exercise comprised a decrease in the P—R interval, an increase in the height of  $P_2$  and  $T_2$ , a diminution of  $R_2$ , and a slight increase in the amplitude of  $Q_2$  and  $S_2$ . These workers pointed out the part which the sympathetic nervous system played in causing these changes and observed that Rothberger and Winterberg (12) obtained the same results by stimulating both stellate ganglia simultaneously. Bazett (13) measured tracings taken during rest and after exercise. His results demonstrated a relative increase in the duration of systole, the P—R interval and the QRS group; the last-named was only slightly prolonged.

These investigations were performed with the heart under the control of the nervous system exerting its normal physiological influence during exercise. In those cases quoted by Rehfisch the conditions were pathological. The object of the work to be described was to determine whether variations in the E.C.G. occurred as a result of increasing the work of the cardiac muscle when nervous influences were partially or completely eliminated.

*Methods.*—Experiments were performed on dogs under complete anaesthesia produced by chloralose (1 decigramme per kilo body-weight injected intravenously). A preliminary dose of morphia was given; chloroform and ether mixture was administered until the chloralose had been run in. Experiments were carried out on the "whole" animal and on the heart-lung preparation.

*Heart-lung Preparation.*—The preparation was made according to the method of Knowlton and Starling (14), the clotting of the blood being prevented by defibrination. In experiments 1 to 3 hirudin was used to prevent blood clotting. In all experiments blood obtained from another animal was added to the artificial circulation. Indirect leads were used in all experiments; the electrodes consisted of copper discs stitched under the skin, as described by Lewis, Meakins and White (15). The curves were carefully standardised in the usual manner; in general Leads I and III were taken but in a few cases only Lead II. In two experiments Leads I and III were recorded simul-

taneously with a double string carrier. The mean arterial blood-pressure was registered by means of a damped mercury manometer writing on a smoked surface, or with a damped Hürthle manometer, the movements of which were photographed simultaneously with the E.C.G. A motor running synchronously with a tuning-fork actuated the time-marker; the disc attached to the motor cut off the light at intervals of 0.18 sec. This time-marker was calibrated against three separate tuning-forks. The photographic plates were measured with the aid of a Lucas comparator, Lead III being selected except where otherwise stated.

The values in the Tables are the average of three heart cycles. The P—R intervals in a number of cases were measured from the peak of the P wave when it was sharply defined; the values in each experiment are therefore relative, not absolute.

The heart-lung preparation was adjusted with a moderate venous inflow in most of the experiments, the artificial resistance (A.R.) being sufficiently high to maintain a mean arterial blood-pressure of 80 to 100 mm. Hg. When the preparation had been running under constant conditions for about 20 minutes, electrocardiographic and blood-pressure tracings were taken. Variations in the amount of work performed by the heart were effected by altering the venous inflow or the artificial resistance. Records were again taken under the new conditions after two to five minutes interval. The mechanical conditions of the circulation were then adjusted to their initial value as nearly as possible and further tracings obtained.

"*Whole Animal.*"—Alterations in the mechanical conditions of the circulation of the "whole" animal were produced by partially clamping the systemic aorta just distal to the left subclavian artery, immediately above the diaphragm or just below the same muscle. The last-mentioned method afforded the opportunity of recording the electrical changes with the chest unopened. With the exception of one experiment, the vagi were cut across in order that alterations in the heart rate might not affect the various factors of the electrocardiogram. In two animals both vagi were cut and both stellate ganglia extirpated. The procedure adopted for obtaining electrical and blood-pressure records was the same as that used in the heart-lung preparation experiments.

#### RESULTS. HEART-LUNG EXPERIMENTS.

The conventional signs  $R_1$ ,  $R_2$ , etc., will be used to denote R in Lead I and R in Lead III respectively. An upright T wave has been termed positive, an inverted T wave, negative. A T wave originally inverted which has the



amount of inversion decreased by experimental procedures, has been said to become less negative.

(a) *Effect of Venous Filling.*

Knowlton and Starling have shown that an increase in venous filling slightly raises the mean arterial blood pressure of the heart-lung preparation when the artificial resistance is kept constant. The form of the E.C.G. has not been determined with varying rates of venous filling and a constant mean arterial blood-pressure, since precautions were not taken to balance the artificial resistance. With a simple increase in venous inflow the P—R and QRS durations may be slightly increased or unaltered. The form of the E.C.G. underwent small variations which were not constant from one animal to another (fig. 1, Observations 7 and 8).

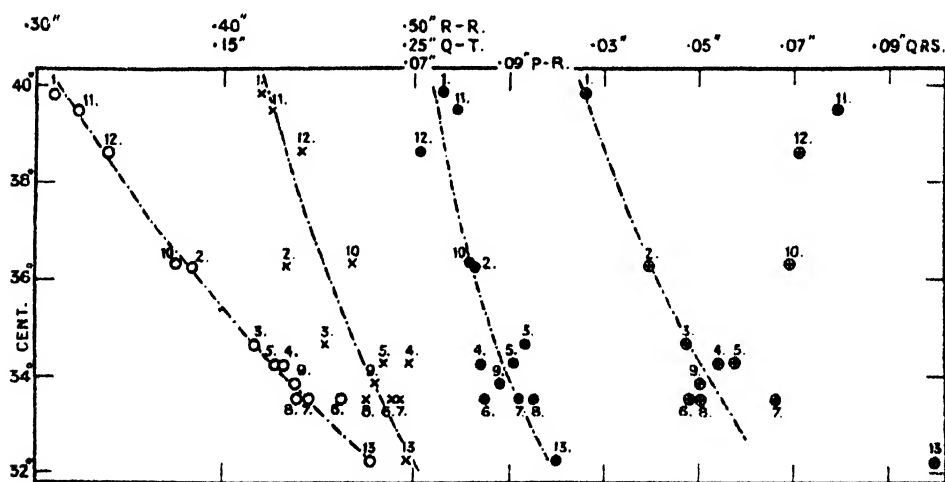


FIG. 1.—O = length of cycle (R—R); X = Q—T duration; ● = P—R interval; ⊕ = QRS duration. The numbers attached to the symbols denote the order in which the observations were made. 1 at 12.15 P.M.; 5 at 12.39 P.M.; 9 at 12.53 P.M.; 13 at 1:24 P.M. Observations 2, 3, 5 and 8 with M.B.P. of 125 mm. Hg. and over. Observations 7 and 8, Peripheral output = 730 c.c. per minute.

(b) *Effect of Increasing the Artificial Resistance.*

Curves taken before, during, and after a rise in mean arterial blood-pressure demonstrated no constant alterations in P, Q, R, S, and T. Table II shows the degree and direction of the changes observed in five experiments selected for their variability. The P—R interval and QRS duration tend to lengthen out very slightly with the higher artificial resistance; in two experiments it

Table II.—Heart-Lung Preparation. Effect of Increasing the Artificial Resistance.

Expt.	R—R	P—B	Q R S	Q—T	P <sub>1</sub>	P <sub>2</sub>	Q <sub>1</sub>	Q <sub>2</sub>	R <sub>1</sub>	R <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>	Per Output c.c. per min.	A.R.	Temp. Cent.	M.B.P.	V.I.	
1*	0.394	0.074	0.027	0.237	—	1.0	—	0	—	3.5	—	0	—	—	+1.0	570	—	—	54	L
	0.390	0.080	0.046	0.225	—	2.0	—	0	—	5.5	—	0	—	—	+3.5	—	—	—	168	L
	0.385	0.073	0.022	0.212	—	1.5	—	0	—	4.5	—	0	—	—	+2.0	—	—	—	90	L
3*	0.445	0.092	0.066	0.239	—	2.0	—	7.0	—	10.0	—	0	—	—	-5.5	730	—	33.5°	84	L
	0.438	0.095	0.050	0.224	—	1.5	—	6.0	—	8.0	—	0	—	—	-7.0	—	—	R	150	L
12	0.437	0.088	0.050	0.228	—	1.5	—	3.5	—	7.0	—	0	—	—	-3.0	290	—	33.8°	120	M
	0.465	0.086	0.070†	0.218?	—	—	1.0	0	5.0	11.0	1.0	0	-4.5	-1.0	125	60	36.4	96	S	
14a	0.509	0.075	0.074	0.246	—	—	0	0	5.5	11.0	1.0	0	-3.0	-2.0	100	R	35.0	170	S	
	0.512	0.082	0.043	0.275	2.0	1.5	0	0	4.5	22.0	0	7.0	d	+7.0	160	70	33.0	105	S	
b	0.538	0.092	0.052	0.269	1.5	0.5	0	0	4.0	17.0	0	0	d	+4.0	—	R	—	180	S	
c	0.523	0.085	0.043	0.278	2.0	1.0	0	0	3.0	20.0	0	7.5	d	+5.0	200	65	33.0	95	S	
	0.575	0.101	0.074	0.300	2.5	3.0	0.5	0	7.5	17.0	2.5	3.5	-4.5	-2.5	240	45	34.7	80	M	
15	0.581	0.100	0.079	0.309	3.0	3.0	1.0	0	7.5	18.5	2.0	?	-4.0	-2.5	—	100	34.6	123	M	
4*	0.577	0.091	0.068	0.296	2.5	3.0	1.0	0	7.5	17.0	2.0	?	-4.5	-2.5	260	40	34.7	85	M	
	0.408	0.124	0.066	0.244	—	—	0	0	—	10.0	—	0	—	-2.5	360	—	35.5	80	M	
	0.401	0.124	0.063	0.229	—	—	0	0	—	12.5	—	0	—	-2.0	—	—	35.5	160	M	
	0.403	0.125	0.068	0.250	—	—	0	0	—	10.5	—	0	—	-3.0	—	—	35.5	45	M	

A.R. = Artificial Resistance.

L = Large.

M = Moderate.

R = Raised.

S = Small.

V.I. = Venous Inflow.

d = diphasic.

\* = Lead II.

† = R downstroke gradually tailing off.

will be noted that there is a small decrease of the P—R interval in one and of the QRS duration in the other (Experiments 3, 12).

With regard to the total duration of the electrical response, it was expected that an increase in the arterial resistance would prolong the total duration of the E.C.G., since Patterson, Piper and Starling (16) found an increase in the duration of the period of contractile stress of the heart under similar conditions. In one experiment these authors noted that the relative durations of the ventricular contractions at 68 and 166 mm. Hg. were 0.224 sec. or 46 per cent. and 0.262 sec. or 54 per cent. of the total cycle respectively. The duration of the electrical response showed no such increase when the arterial resistance was raised, in fact at the more frequent heart rates it became slightly diminished. No great stress can be laid on these measurements because of the difficulty in estimating the exact end of the T-wave. The result of one experiment in which great care was taken to keep the temperature constant is given at the end of Table II, the diminution in the Q—T duration amounts to 0.015 sec.

*(c) Influence of the Elasticity of the Peripheral Circulation.*

Since the E.C.G. of the heart-lung preparation was not markedly changed by the alterations in the mechanical conditions already described, further experiments were performed in order to discover whether the elasticity of the peripheral circulation affected the E.C.G. Apparatus was set up whereby it was possible to change over the normal heart-lung circulation to one which had excessive elastic properties or to one with a rigid tube peripheral circulation. The scheme of the circulation is pictured in fig. 2. The air reservoir A had a capacity of 220 c.c. and was closed at one end by a tightly stretched rubber membrane 4.5 cm. in diameter. When this reservoir was in circuit the blood was seen to oscillate in a marked manner in the arterial cannula at each cardiac systole and diastole. Galvanometric curves were obtained in two experiments with these modified conditions of the heart-lung circulation.

Changing over from the heart-lung circulation to the rigid tube system or to the excessively elastic circulation produced no alterations in the E.C.G.

*(d) The Influence of the Size of the Aortic Cannula.*

Wiggers (17) has raised an objection to the heart-lung circulation in that the cannula in the brachio-cephalic artery produces conditions similar to an aortic stenosis. However this may be, it was found that in two experiments the passage of the blood through a cannula in the thoracic aorta instead of

through the brachio-cephalic artery was without effect on the form of the E.C.G. The scheme of the circulation is shown in fig. 2.

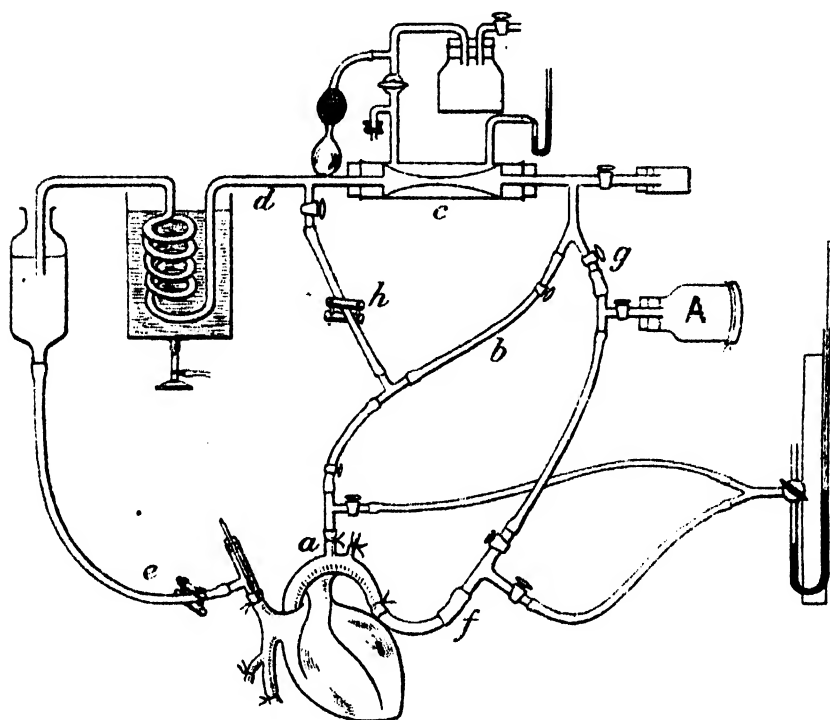


FIG. 2.—Circulation scheme for varying the elasticity of the peripheral resistance. The course of the blood through the peripheral resistance for the different systems is shown by the letters :—

1. Normal heart-lung preparation, *a, b, c, d, e*.
2. Circulation with excessive elastic properties, *a, b, g, A, c, d, e*.
3. Rigid tube peripheral resistance, *a, h, d, e*. The arterial pressure was controlled by a clamp on the pressure tubing at *h*.
4. Circulation through the systemic aorta, *a, f, g, c, d, e*.

In the actual experiments clamps were used instead of the stop-cocks shown in the figure.

### RESULTS OF EXPERIMENTS ON THE "WHOLE" ANIMAL.

#### (a) *The Effect of Partial Compression of the Aorta High Up.*

The screw-clamp was applied to the aorta just distal to the origin of the left sub-clavian artery. The changes observed in the E.C.G. are given in Table III, from which it will be seen that the main alterations observed are that  $R_s$  increases in amplitude,  $T_1$  and  $T_2$  tend to become more positive if initially positive, or less negative if initially negative. The P—R and QRS



0.366	0.065	0.040	0.197	1.0	3.0	0	0	9.0	10.0	0	3.0	0	+2.0	180	Normal E.C.G. Vagi intact. After vagal section. Low compression above diaphragm. Four minutes later. Before high compression. Nine cycles after clamp was screwed up. Ten minutes after release of clamp. After removal of both stellate ganglia. Just before high compression. Nine cycles after clamp was screwed up.
0.419	0.068	0.044	0.217	1.5	4.5	0	0	6.0	30.5	0	8.0	-1.0	0	155	
0.515	0.086	0.045	0.227	1.0	4.0	0	0	5.0	27.0	0	8.5	-1.0	+3.0	170	
0.389	0.068	0.041	0.214	2.0	5.0	0	0	7.0	24.0	0	8.5	-2.0	+2.0	130	
0.398	0.065	0.043	—	—	4.0	—	0	—	24.0	—	8.0	—	+2.5	140	
0.410	0.070	0.043	—	—	5.0	—	0	—	34.0	—	8.0	—	+2.5	180	
0.387	0.062	0.047	0.221	1.5	5.0	0	0	6.5	25.0	0	8.0	-2.0	+3.0	150	
0.531	0.091	0.045	0.249	2.5	4.0	0	0	8.0	25.0	0	8.0	-2.0	-3.0	150	
0.567	0.095	0.044	0.276	—	4.0	0	0	—	24.5	0	5.0	—	-4.0	120	
0.602	0.093	0.044	0.275	—	4.0	0	0	—	27.0	0	5.0	—	-3.5	160	

durations are slightly prolonged, the maximum in each instance being 0.005 sec. Individual variations occur, but the changes in  $R_s$ ,  $T_1$  and  $T_s$  are definite. Fig. 3 (Plate 18) shows the E.C.G. in Experiment 20.

(b) *The Effect of Partial Compression of the Aorta Low Down.*

In these experiments the clamp was applied to the aorta just above the diaphragm.  $P_1$ ,  $P_s$  and  $R_1$  diminished slightly, and  $T_s$  was inconstant, but in the majority of experiments it moved upwards (Table III). Except in Experiment 26 the P—R and QRS durations are only slightly increased. Compression of the aorta below the diaphragm in two experiments had no obvious effect on the E.C.G.\*

CONSIDERATION OF RESULTS.

From the foregoing description of the experiments and results of the effect on the E.C.G. of increasing the amount of work performed by the heart under different mechanical conditions of the circulation, it is evident that the amount of work does not determine the form of the E.C.G. It would appear that the shape of the E.C.G. is related to the method adopted for increasing the cardiac work. This being so, it was necessary to search for some cause which might influence the E.C.G. and which might be governed by the experimental procedure involved. It had been noticed that with the various methods used for raising the blood-pressure the heart frequency showed alterations which were not constant; the changes were slight, but occurred even in the experiments on the completely denervated heart. Attention was therefore turned to the control of the blood temperature. It was not so much the slight temperature changes of the blood in the heart which appeared to be of importance as any alteration in the normal difference of temperature between the right and left hearts. Claude Bernard (18) has shown that this temperature difference is normally about  $0.2^\circ \text{C}$ ., the temperature of the right heart being the higher.

In order to ascertain the importance of this factor in determining the form of the E.C.G., experiments were carried out on the "whole" animal and on the heart-lung preparation.

\* Langley, McSwiney, Mucklow, Stopford, and Wilson, on clamping the aorta above the diaphragm, found no change in the E.C.G. ('Proc. of Physiol. Soc.,' November 18, 1922).

#### CONTROL OF TEMPERATURE CHANGES.

The temperature of the blood was taken on each side of the heart before, during and after partial compression of the aorta in the "whole" animal, and before, during and after a rise in the artificial resistance in the heart-lung preparation. Thermometers 44 cm. in length, 4 mm. in bore and graduated to  $0.1^{\circ}$  Centigrade, were inserted into the right and left hearts through the right external jugular vein and the left carotid artery respectively. In one experiment the bulb of the thermometer was not able to be pushed into the left ventricle, it remained in the brachio-cephalic artery. In another experiment the bulb of the thermometer rested in the aorta. Other experiments were performed with the thermometers in the right and left auricles. In some cases adrenaline was injected intravenously. The animal was kept warm with hot-water bottles and the artificial respiration apparatus pumped in warm air; a change in the temperature of the respired air did not affect the main results.

Table IV gives the data of two experiments; four others produced similar results. It is obvious that a fall of temperature of  $0.10^{\circ}$  to  $0.30^{\circ}$  C. on both sides of the heart is caused when the blood-pressure is raised by partial compression of the aorta or by injection of adrenaline. The same order of change was observed in the heart-lung preparation on raising the artificial resistance.

The explanation of the fall in temperature on both sides of the heart with the means adopted for raising the blood-pressure seems to be that a greater proportion of blood will pass through the head, neck and upper limbs, which are relatively colder than the abdominal area. This temperature difference will be exaggerated if hot water bottles are placed on the abdomen. With regard to the temperature fall of the blood in the heart observed after adrenalin chloride injection, the blood redistribution effect is more complicated, but probably the cold peripheral blood exerts its temperature effect on the mixed blood in the right heart in a somewhat similar manner. With the method of temperature registration adopted the times of appearance of the temperature changes on each side of the heart could not be compared with any great certainty. In the case of the heart-lung preparation the fall in temperature is due to the diminished peripheral output, and therefore a diminished flow of warm blood from the reservoir entering the right auricle. It was necessary therefore to determine whether such alterations in the blood temperature within the heart, and perhaps slight differences in the normal temperatures on



Table IV.

	Temp. C°.		Heart Rate.	M.B.P.
	L.A.	R.A.		
(1.) Dog. ♀ 10.0 kilo. Vagi Cut. Temp. of Respired Air 31.0° C.				
Adrenalin chlor. 1 : 10,000 m V. intrav.	33.80	33.95	141	120
40 sec. after adrenalin ....	33.70	33.95	162	200
1 min. 40 sec. ditto ....	33.50	33.80	132	—
3 min. ditto ....	33.60	33.95	126	160
3 min. 30 sec. ditto ....	33.80	34.05	129	130
	Temp. C°.		Heart Rate.	M.B.P.*
	Aorta.	R.V.		
(2) Dog ♀ 10.25 kilo. Vagi Intact, Chest closed. Respired Air 35.5° C.				
	34.40	35.95	186	100
Partial compression of abdominal aorta immediately below the diaphragm for 90 sec.	34.40	35.95	—	20
	34.20	35.85	—	36
	34.20	35.80	—	40
	34.20	35.85	180	100
	34.30	35.90	—	100
1 min. after clamp release ....	34.40	35.95	—	104

L.A. = Left Auricle. R.A. = Right Auricle. R.V. = Right Ventricle.

\* Manometer in femoral artery.

The temperatures of the blood in the aorta and in the R.V. are not absolute values in Experiment 2.

each side of the heart, would influence the form of the E.C.G. For this purpose a modified form of heart-lung preparation was set up, whereby the temperature of the blood on each side of the heart could be altered at will.

#### INDEPENDENT RIGHT AND LEFT-HEART CIRCULATION.

*Method.*—A heart-lung preparation was made and then the modified form of the circulation, as described by de Barenne (19), connected up. In this form of preparation the blood passes from the venous reservoir to the pulmonary artery, through the lungs, left auricle, left ventricle, aorta, and back again to the venous reservoir, after being warmed in the usual manner. The blood from the coronary circuit is collected by means of the cannula inserted into the

superior vena cava, and measured under different mechanical conditions of the circulation. The work of the right heart under these conditions is practically *nil*. In the further alteration of the system devised for the investigation of varying the temperature on each side of the heart, the procedure was as follows. A cannula was placed in the proximal end of the pulmonary artery and the superior vena cava connected to a second glass reservoir, A, fig. 4. The blood from the coronary circulation, instead of flowing out of the superior vena cava as in the Barenne modification, passed to the right

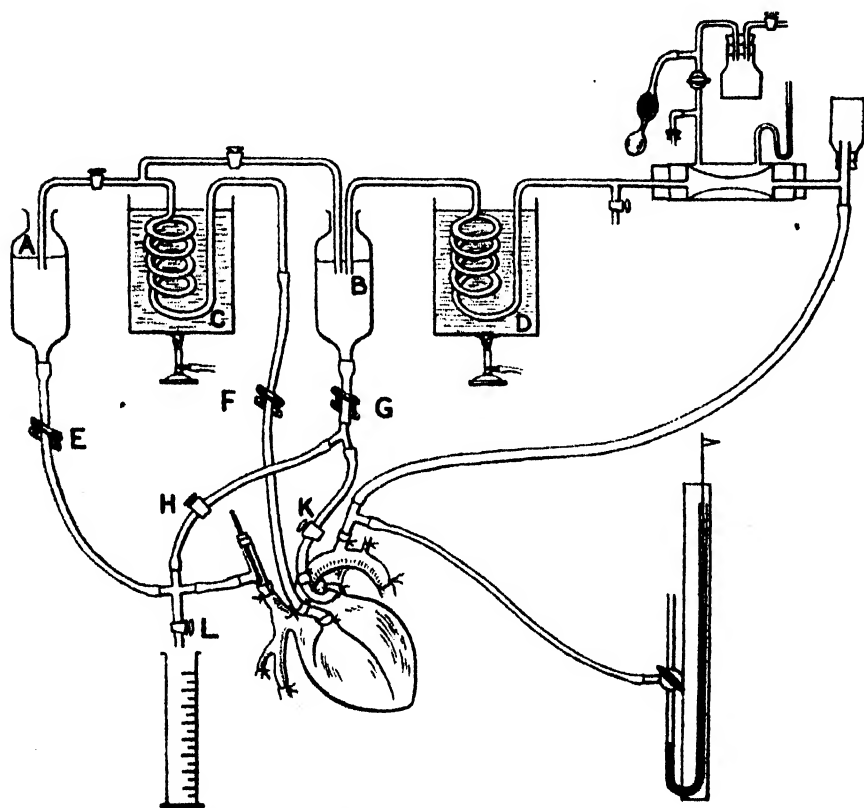


FIG. 4.—Scheme of the circulation used for independent right and left-heart circulation. (For explanation see text.)

ventricle and was ejected into the pulmonary artery, eventually arriving at the second reservoir (A) after passage through the thermostat (C). Additional blood was added to this reservoir and the venous inflow controlled by a screw clip (E) on the rubber tubing. An artificial resistance was not inserted distal

to the pulmonary artery since the height of the thermostat above the heart was sufficient to maintain the blood-pressure at a normal value. A screw clip (F) was placed on the tubing connected to the pulmonary artery for raising the arterial resistance. In order to complete the left-heart circulation the venous reservoir (B) was switched over to the distal end of the pulmonary artery. The right and left-heart circulations were then independent. It is true that the anatomical arrangement of the coronary vessels allows some of the blood from the left-side circulation to enter into the right-heart, and for this reason the blood from the left-heart reservoir gradually diminishes and that in the right-heart reservoir increases. In order to keep the amount of blood in each reservoir approximately constant, a glass T-piece was inserted into the rubber tube leading from the thermostat (C), which enabled the blood to be by-passed into the reservoir (B) when desired. The escape of the blood from one to the other side of the circulation did not materially affect the control of temperature changes of the blood entering the two auricles.

Electrocardiograms were obtained with the Barenne circulation, where the right ventricle is performing little or no work, and with the apparatus for the independent right and left-heart circulation when the difference in temperature on each side was well marked.

*Results.*—The first two experiments in Table V show the change in the E.C.G. due to the Barenne circulation. The QRS duration increased in both experiments and the P—R interval is prolonged in Experiment 14, in which the Barenne circulation had been running one hour when the last reading was taken. The effect on the T-waves of the Barenne circulation is the same in both experiments— $T_1$  became more negative and  $T_2$  more positive (fig. 6, Plate 19).

From Experiment 17 one sees that a difference in temperature of  $3^{\circ}$  C. between each side of the heart has no marked effect on the E.C.G., therefore variations of  $0.3^{\circ}$  C. which were met with on raising the mean arterial blood pressure in the earlier experiments may be neglected.

Adrenaline injected into the right-heart circulation only had an interesting effect on the E.C.G. (fig. 5, c, Plate 19). Lead III is very similar to the type of curve obtained by Rothberger and Winterberg (12) on stimulation of the right stellate ganglion.

Although the addition of adrenaline was carried out in one experiment only, the result suggests that adrenaline is able to exert its effect on the endocardial surface of the ventricle and that the sympathetic nerve supply to the

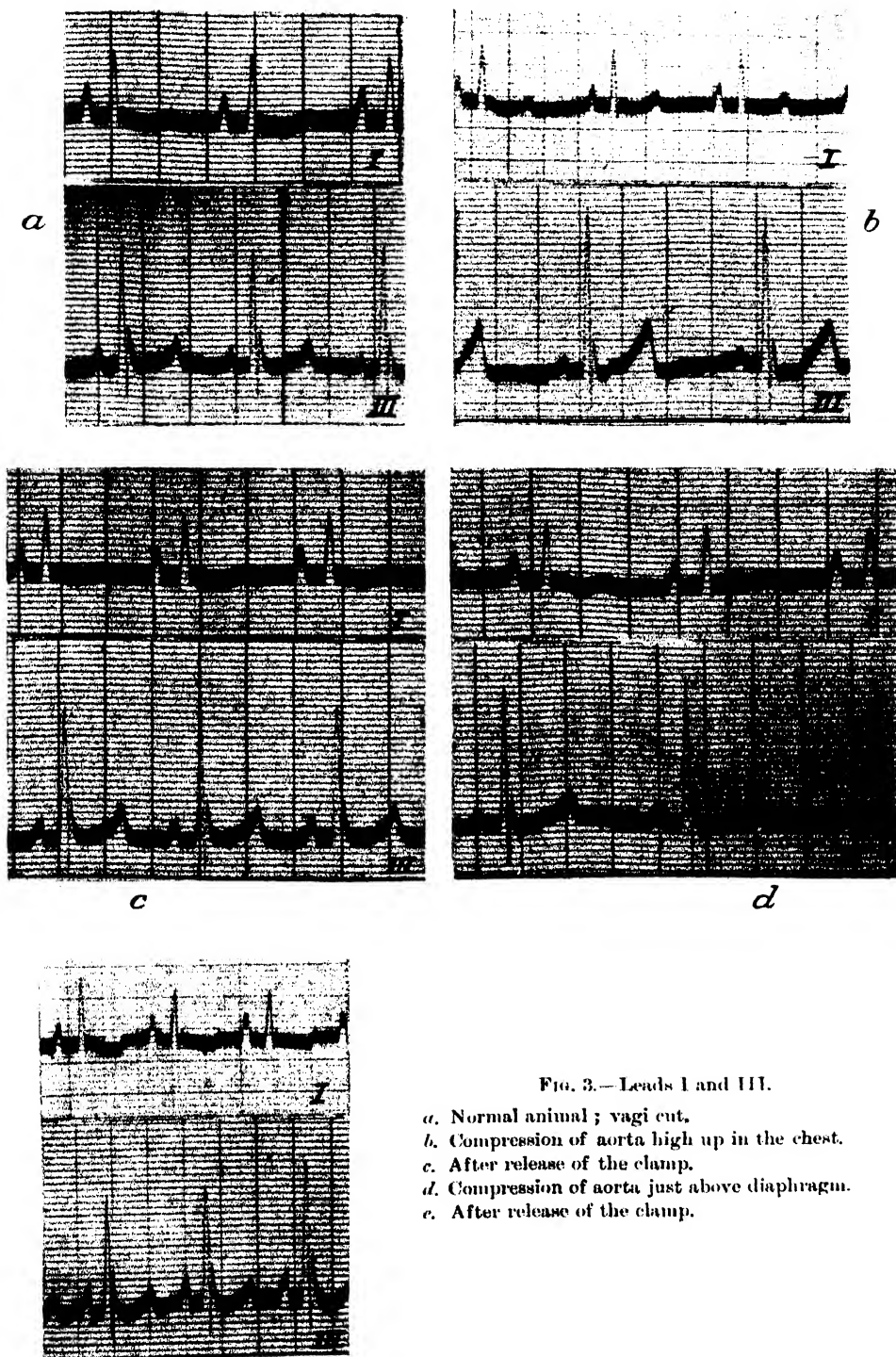


FIG. 3.—Leads I and III.

- a. Normal animal; vagi cut.
- b. Compression of aorta high up in the chest.
- c. After release of the clamp.
- d. Compression of aorta just above diaphragm.
- e. After release of the clamp.

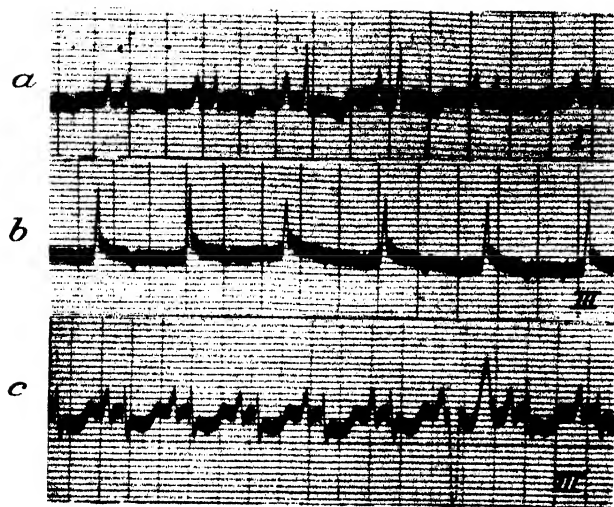


FIG. 5.—*a*, *b*, Leads I and III of the independent right and left heart circulation. *c*, Effect of adrenaline injected into the right heart circulation only, Lead III.

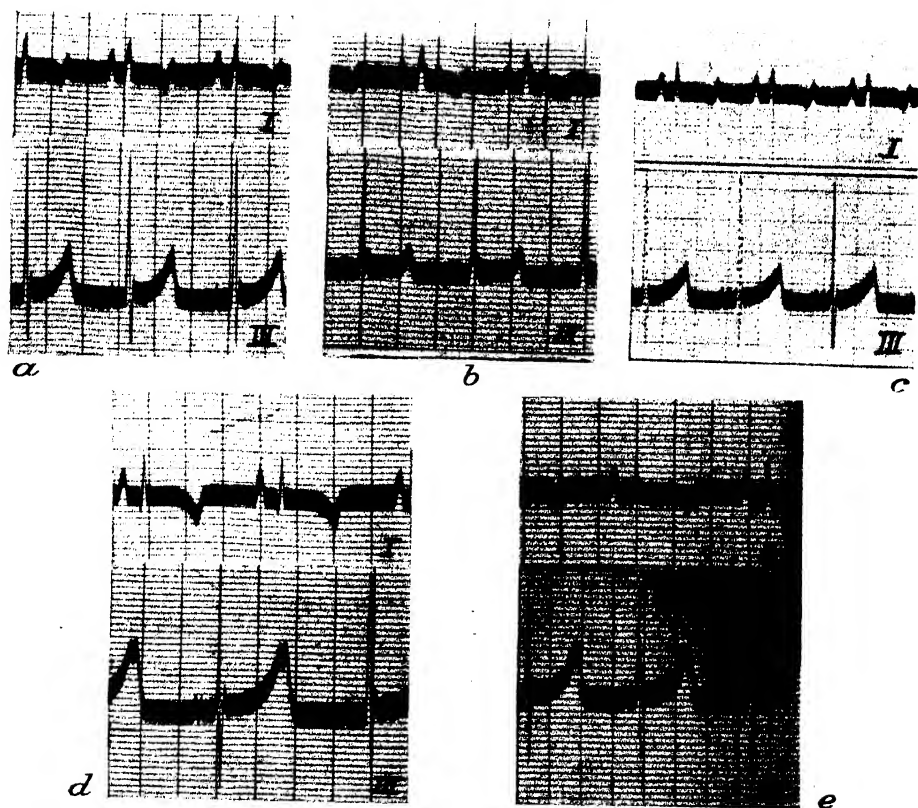


FIG. 6.—Leads I and III. *a*, Heart-lung preparation (see Table II, 14*a*). *b*, Heart-lung preparation. Normal. Artificial resistance raised (see Table II, 14*b*). *c*, Heart-lung preparation. Return to normal conditions (see Table II, 14*c*). *d*, Barenne preparation (see Table V, 14*c*). *e*, Barenne preparation (see Table V, 14*d*).

Table V.—De Barenne Circulation and the Independent Right and Left-Heart Circulation.

Expt.	R-R	P-R	Q-RS	Q-T	P <sub>r</sub>	Q <sub>r</sub>	R <sub>r</sub>	S <sub>r</sub>	S <sub>i</sub>	T <sub>r</sub>	T <sub>i</sub>	M.R.P. A.R.	Indov.	Peripheral Output c.c. per min.	Coronary Output c.c. per min.	Total Output c.c. per min.	T° of S.V.C.	T° of L.V.	
15	0.419	0.035	0.043	—	2.0	2.0	0	14.0	8.0	0	8.0	-2.0	+2.0				36.2		Normal
	0.321	0.102	0.409	—	3.5	2.5	0	13.0	9.0	0	8.0	-2.0	-2.0				—		Heart-lung preparation.
	0.406	0.099	0.059	—		0	0	8.0	6.0	0	?	-5.0	+2.0						Barrenne modification.
16	0.523	0.056	0.043	0.275	2.0	1.0	0	3.0	19.5	0	7.0	d	+5.0				35.5		Heart-lung preparation.
	0.465	0.102	0.045		2.5	1.0	0	5.0	22.0	0	7.0	-3.0	+9.0				34.8		Barrenne modification.
	0.700	0.105	0.047	3.0	1.5	1.5	0	6.0	23.0	0	11.0	-3.5	+10.0				—		ditto.
	0.664	0.106	0.050	2.5	?	1.5	0	5.0	16.0	0	9.0	-2.0	+7.5				33.2		ditto.
	0.596	0.106	0.062	2.0	?	1.5	0	5.5	16.0	0	9.0	-3.0	+6.5				33.6		ditto.
17	0.450	0.076	0.041	0.213	3.5	d	0	5.5	9.5	0	0	-2.0	-1.5				35.5		Normal heart-lung.
	0.535	0.099	0.044	0.357	4.5	-1.5	0	7.0	5.5	0	0	-4.5	-1.5				30.5	33.0	Right and left heart circulation.
	0.605	0.086	0.049	0.265	5.5	-1.5	0	9.5	6.5	0	0	-4.5	-1.5				30.0	30.0	ditto.

T° of S.V.C. = Temperature of blood in the Superior Vena Cava.  
T° of L.V. = Temperature of blood in the Left-Heart Reservoir.

heart is homolateral. It is evident that this experiment must be repeated before one can arrive at a final decision.

The coronary flow in Experiment 14 was excessive and on measurement was found to be equal to the peripheral outflow. No explanation of the phenomenon was discovered.

#### DISCUSSION.

In the heart-lung preparation *in situ* the reaction of the E.C.G. to changes in the mechanical conditions of the circulation are negligible and are not comparable with the muscular reactions which Patterson, Piper and Starling found under similar conditions. The path of the excitation wave, therefore, is the same under various conditions of contractile stress. On the other hand, the E.C.G. of the denervated heart of the whole animal undergoes changes when the mean arterial pressure is raised by partial compression of the arch of the aorta. Partial compression of the aorta lower down produces only a slight or no change in the E.C.G., although the mean arterial blood-pressure may reach the same height as in the high compression.

In the heart-lung preparation it is possible to alter the E.C.G. by conditions which affect the work of the two sides of the heart in different directions.

Under these experimental conditions an endeavour has been made to separate as far as possible the effects on the electrocardiogram of the work performed by each ventricle. It is evident that alterations in the work performance of the left ventricle will not leave the right ventricle unaffected. It is for this reason that the results obtained must be interpreted with caution, and the more so since the mode of contractile response of the whole heart has been throughout these experiments an unknown factor.

The most striking change in the electrocardiogram during increased work of the heart muscle occurred when in the "whole" animal the aorta was partially compressed just distal to the left sub-clavian artery.  $R_s$ ,  $T_1$  and  $T_3$  were increased in amplitude, but in a few experiments these alterations failed to appear. The augmentation of R and T observed by other workers has already been mentioned. That  $R_s$ ,  $T_1$  and  $T_3$  become larger when the work performance of the heart is increased by certain methods is also true for the completely denervated heart or the heart with vagal influences removed. The evidence for this effect being a left ventricular one is uncertain but suggestive.

The Barenne heart-lung preparation and the accompanying electrocardiograms point to an increase in positivity of  $T_s$  and an increased negativity

of  $T_1$  as taking place when the left ventricle performs work in excess (relative to the normal heart) of that of the right ventricle. In addition two experiments carried out, in which the pulmonary artery was partially occluded, showed in one experiment  $T_3$  more negative during application of the clamp, and less negative than the original value on release of the clamp. In the other experiment  $T_3$  underwent no change when the clamp was partially screwed up but became more positive on release. If one takes into account that there is a sudden flow of blood into the left-heart on removal of the clamp, then the work of the left-heart is increased and that of the right-heart diminished. Thus, when the work of the left ventricle was increased after release of the pulmonary artery clamp,  $T_3$  became more positive in one and less negative in the other experiment. All the experiments taken together suggest that when the work of the left-heart is increased out of proportion to that of the right-heart,  $T_3$  becomes more upright whatever its initial value. This statement will be subject to the reservation that the left ventricle is responding to work in a manner which is as yet undetermined, and indeed may not be the normal physiological response, for we have seen that under other conditions of augmented work, no such changes in the electrocardiogram are observed. Whether the alterations in  $T_3$  are actually the result of the adjustment of tension differences during contraction or of the increased coronary blood flow which will take place, is at present problematic, although the experiments with the Barenue circulation show that the former at least play a part.

#### CONCLUSIONS.

1. The contractile and electrical response of the isolated ventricle of the tortoise (*Testudo graeca*) beating under isometric conditions has been measured with varying degrees of cardiac filling. As the filling is increased, the duration of rising tension, the initial tension, the maximum tension developed, the duration of muscular relaxation, and the duration of electrical response are all increased; the height of the electrical response is diminished.
2. In contradistinction to the muscular reactions of the heart-lung preparation to increased work the E.C.G. undergoes no change.
3. Alterations in the E.C.G. of the denervated heart of the whole animal under certain conditions of increased cardiac work are described.
4. A new modification of the heart-lung preparation is described in which it is possible to vary independently the work of each side of the heart.
5. The relation of changes in the electrocardiogram to the relative amount of



work performed by each side of the heart under experimental conditions is discussed.

I am greatly indebted to Professor Starling for his criticism and advice throughout the investigation.

The expenses of this research were defrayed by grants from the Royal Society and the British Medical Association, to whom I express my thanks.

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*A Comparison between Certain Features of the Spinal Flexor Reflex and of the Decerebrate Extensor Reflex respectively.*

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(1)

We described recently a difference between the spinal reflex of flexor muscles of knee and ankle on the one hand and the decerebrate crossed reflex of the knee extensor on the other in regard to transmission of the rhythm of the exciting stimulus applied to the afferent nerve. Another difference between these two types of reflexes is exhibited in the form of curve of tension-development exhibited in isometric myograms of their tetani. Examination of these shows a divergence which cannot be accounted for by such differences as exist between the tetanic behaviour of the several muscles or motor nerves themselves.

I.—*Method.*

The method employed has been the same as in our previous paper. All muscles except that one whose contraction was to be recorded have been paralysed by nerve-section or resection; and the myograph has been of the isometric type with optical registration. As stimuli series of single shocks separated from an inductorium usually coreless have, by means of the vibrating key described in the previous paper, been delivered to the suitably bared afferent or efferent nerve, the anode for the shock being always towards the cut end of the nerve. The distance between the electrodes on the nerve has been 12 mm. (2). Occasionally, the stimuli have been applied by a stigmatic kathode electrode in the skin. The preparation (cat) has been decerebrate, all of the brain anterior to the posterior colliculi being removed. For purely spinal reflexes, the spinal cord has, in addition, been transected either immediately or some days or weeks prior to the decerebration; the level of spinal transection has varied in different experiments between 12th thoracic and 3rd lumbar segments.

II.—*Tetani provoked by Direct Stimulation of the Motor Nerve.*

These may be briefly designated mn. tetani. For comparison with the reflex tetani of the same muscles, we have observed mn. tetani in semi-tendinosus and tibialis anticus (flexors) and vasto crureus and rectus femoris

(extensors), and with the same myograph and modes of stimulation and in the same experiment as that which yielded the reflexes. For descriptive convenience, that part of the tetanus which corresponds with the fully summed contraction tension is termed the *plateau*; the curve of increasing tension by which, starting from outset of the contraction, the plateau is reached may be termed the *ascent*. The bend of the curve where ascent merges into plateau, since it requires some special mention, can be termed conveniently the "ascent-plateau turn." The tetanus plateau is reached after a certain number of successive stimuli have been delivered, which number is practically constant for a given muscle and a given frequency and strength of stimulus and a given initial stretch of the resting muscle. In other words, under those conditions the ascent-period is of constant duration: the number of contraction waves in it may be called the "summation-number." Where a stimulus-frequency is chosen somewhat too low for complete fusion of the successive contraction waves the summation-number can be read directly from the myogram. Such incomplete tetani possess this and some other advantages for analysis of the tetanus, and we have employed them generally in preference to complete tetani.

Mn. tetani of *semitendinosus*, *tibialis anticus*, *vasto crureus* and *rectus femoris* do not in our experience when examined under like conditions differ greatly as between these several muscles (1). For all of these muscles the isometric-tetanus myogram-curve conforms to the same well-known type. In our experience, the summation-number tends to be somewhat higher for quadriceps extensor than for the two flexor muscles, and the former's plateau tends more often to give for some distance a rising gradient from the abscissa axis, making the ascent-plateau turn less sharp. But the four muscles extensor and flexor as regards their mn. tetani show under similar conditions of stimulation rate, etc., relatively small departure from one type of isometric tetanus curve common to them all. Such differences as exist between them are slight as compared with the differences we find between the reflex tetani of the two flexors in their ipsilateral spinal reflex on the one hand and the tetani of the decerebrate crossed knee-extensor reflex on the other.

### III.—*Ipsilateral Spinal Flexor-Reflex.*

This we have examined in *semitendinosus*, a knee-flexor, and *tibialis anticus*, an ankle-flexor, and as afferent nerves for the former muscle ipsilateral peroneo-popliteal, for the latter ipsilateral popliteal. The ascent and plateau of the reflex tetanus often resemble closely those of the mn. tetanus of the same

muscles given by stimuli of the same frequency. Sometimes so close is the resemblance that apart from the longer latency of commencement and slower

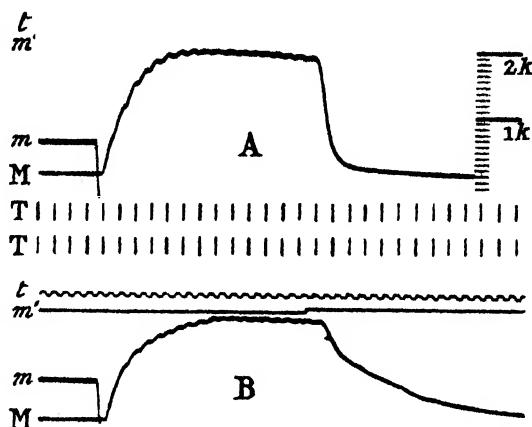


FIG. 1.—*Tibialis anticus*: A, motor nerve stim'd, coil 17 cm. B, afferent nerve, ipsilateral popliteal, stim'd coil 13.8 cm. Stim. freq. 40 p. sec. T, 0.04 sec. Myograph magnification of tendon movement 58 times. Calibration for tension of both at side of A.

[In the figures throughout, M = myograph, its ordinates are verticals, not arcs; T = time;  $t$  = frequency of stimulus;  $m$  = unshorting of stimulating circuit for tetanic series;  $m'$  = shorting of stimulating circuit for tetanic series;  $n$  = unshorting of stimulating circuit for twitch or short series;  $n'$  = shorting of stimulating circuit for twitch or short series;  $z$  = cut-out;  $z'$  = 2nd of same.

The stimulation signals mark the opening and closing of the short-circuit of the stimulating current; they show therefore the actual latencies only to within the period of frequency of repetition of the successive stimuli, e.g. with stimuli at 40 per sec., are exact only to 1/40 sec.

The scale against which tensions are marked is in millimetres and centimetres on the original records. Magnification by myograph refers to size of original record.]

terminal subsidence following cessation of the stimulus which characterise the reflex there may be little to distinguish the reflex from the mn. tetanus. With incompletely fused tetani the number and progressive decrease in height of the successive steps of the ascent, and the degree of discreteness of the waves along the plateau are then in the reflex and the mn. reaction approximately similar.

This argues for the reflex a somewhat unexpected directness of transmission of the centripetal impulses' effect to the motoneurones. In view of it, if we accept identity of reaction for the motor-nerve impulse whether discharged from the centre or excited from the motor-nerve itself (Adrian (16), A. Forbes (17), K. Lucas (16)), the grading of intensity of the reflex contraction may be

regarded as mainly given, in accordance with the views of various observers, by a spatially additive feature in the central mechanism, namely the number of the motoneurones which the reflex activates. This view would place the grading of intensity of reflex contraction of a muscle in so far on the same footing as that given by Adrian (18) and K. Lucas (18) to the grading of the mn. tetanus of the muscle.

That with mn. tetani the stronger contraction evoked by a stronger stimulus as compared with a weaker is due to additional contracting units being engaged by the former, seems supported by the result of abruptly increasing the strength of the break-shocks during a sub-maximal tetanus. The result of this on the mn. tetanus (fig. 2, A) is an incremental contraction whose fresh ascent broadly repeats in character the initial ascent, with a sequence of diminishing contraction-steps up to the new plateau of augmented contraction-tension. And this is so, although the incremented stimulus, if used *ab initio*, gives a tetanus with ascent-steps no more numerous than is usual for that frequency and intensity of stimulus. Similarly, a like experiment with the reflex preparation has a broadly similar result. Thus with semitendinosus the effect of a sudden increment of stimulus to the afferent nerve during a reflex contraction already in operation is a renewal of ascent broadly repeating the features of the initial ascent (fig. 2, B, C) much as in the mn. tetanus. This suggests that the incremental reflex brings into play an additional number of motoneurones. There is, however, a difference between the reflex result and the mn. result, in that on cessation of the incremental stimulus—the initial stimulus remaining still in action—the contraction height (tension) in the mn. tetanus drops at once to or below the original non-incremental value. With the reflex the fall of tension on withdrawal of the incremental stimulus is more gradual, and for some time the contraction, continued under the initial stimulus alone, is of a height above that of the plateau originally given by that stimulus. This is doubtless due to the after-discharge of the incremental reflex; it shows that the reflex units of higher threshold react with after-discharge as do those of lower threshold.

Occasionally, the reflex myogram differs from that of the mn. tetanus, in that its ascent is slightly longer and more gradual than that of the latter. Much more often, however, in our experience it deviates from the mn. curve in the exactly opposite sense. In our experience the most common form of the isometric myogram of the reflex is one which (fig. 6A, fig. 8) presents a steeper ascent and a sharper ascent-plateau turn than does the mn. tetanus itself, when comparison of the two tetani is made with samples of fairly

corresponding plateau-height. This latter type of difference seems, therefore, important as being usual and characteristic. For assessing it some preliminary

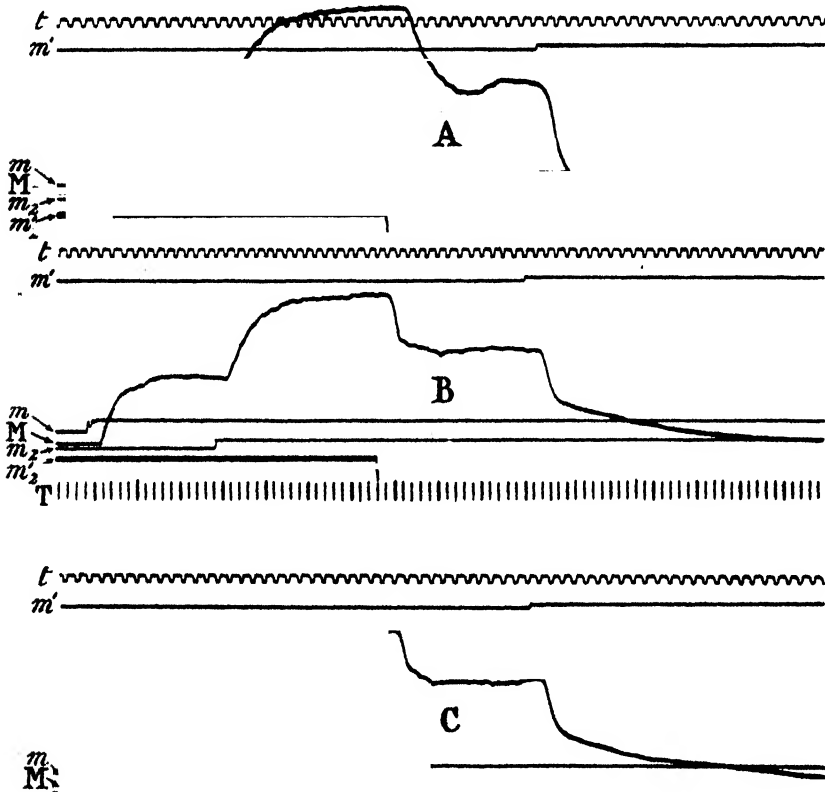


FIG. 2.—*Semitendinosus*: A, motor nerve stim'd, coil 28.5 cm., strength temporarily altered by removing and re-introducing resistance in primary circuit, as shown by signals  $m_1$  and  $m'_1$ . B and C, afferent nerve, ipsilateral peroneopopliteal, stim'd coil 14.7 cm., strength temporarily altered as in A. Stim. freq. 38 p. sec. in all. T, 0.02 sec.

enquiry is required as to how far the mn. tetanus itself conforms with regularity to one standard form in its isometric contraction curve. Its curve is certainly affected by three circumstances: (1) rate of frequency of stimulus, (2) intensity of stimulus, (3) degree of initial passive stretch or tension of the muscle when the tetanus is provoked.

(1) Other conditions being the same, the ascent-curve is steeper, the ascent-time less, the summation number higher, and the ascent-plateau turn sharper

the greater the frequency, within limits, of the serial stimuli employed (fig. 3, A). Thus, in *tibialis anticus* an ascent-time of 0.2 seconds under stimuli at 45 per

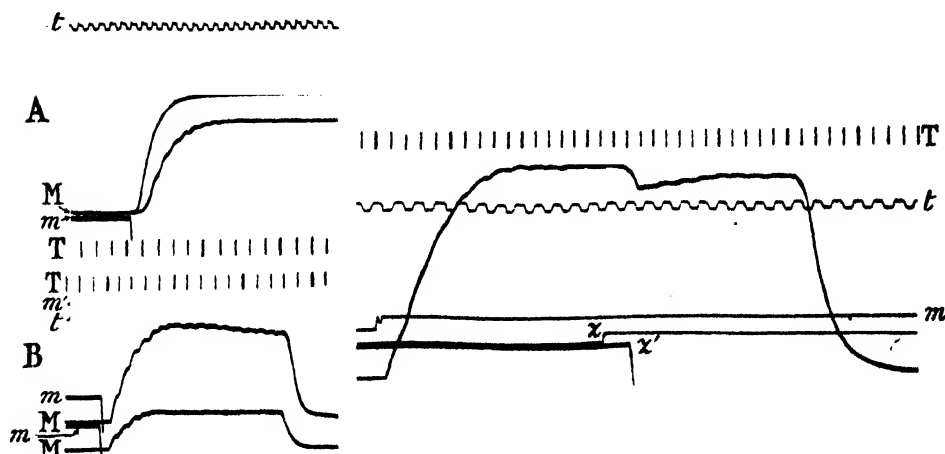


FIG. 3.—A. *Vasto crureus*: motor nerve stim'd, coil 19.5 cm. at 80 p. sec. for higher curve, and for lower curve 40 p. sec., coil 20.5 cm. T, 0.04 sec. B. *Tibialis anticus*: motor nerve stim'd., coil 17 cm. for upper curve; coil 19.5 cm. for lower curve: stim. freq. 40 p. sec. T, 0.04 sec. C. *Semitendinosus*: motor nerve stim'd., coil 26 cm. for top curve, coil 21.8 cm. for bottom curve, one shock cut out (see Signals), giving drop in plateau followed by slow recovery. Stim. freq. for both curves 39 p. sec. T, 0.02 sec.

second became an ascent-time of about 0.1 second on increasing the stimulus frequency to 90 per second. Again, with the *vasto-crureus* an ascent-time of 0.2 second given by stimuli at 40 per second became an ascent-time of 0.12 second when the stimulus frequency was increased to 80 per second. And a similar influence of stimulus frequency is easily verified in the tetani of *semitendinosus*. But this influence, although important in its general bearing, does not explain the sharper rise and bend of the reflex than of the mn. tetanus as dealt with here, because that difference is observed when the stimulus frequency is the same both for reflex and for mn. tetanus.

(2) Other conditions remaining the same, the ascent-curve of the mn. tetanus is shorter, the summation-number smaller, and the ascent-plateau bend often sharper, for minimal and weak sub-maximal tetani than for strong sub-maximal and maximal tetani (fig. 3, B, C; and fig. 4). The accompanying Table exemplifies this from all three types of mn. preparation we have used. It confirms for these mammalian muscles a point already noted (Kohnstamm) (3) in observations on the isometric tetani of the excised *gastrocnemius* of the frog.





Table I.

Muscle.	Bk. sks. per second.	Plateau height (Tension).	Steps to Plateau.	Stim. strength.
		mm.		cm.
Tibialis anticus .....	38	7	5	19.5
" .....	38	17	6	18
" .....	38	22	7	17
Semitendinosus .....	56	16	5	15
" .....	56	38	9	12.75
" .....	56	46	10	12
" .....	37	24	6	14.5
" .....	37	33.5	10	12.5
Vasto crureus .....	36	25	8	24.2
" .....	36	46	13	24.2

Core-  
less  
coil.

(3) Both with semitendinosus and with tibialis anticus increase of initial passive stretch very distinctly affects the form of the myogram in the following ways. In mn. tetani, examined in one and the same muscle and under stimulation of the same intensity and frequency, the ascent curve of the tetanus when provoked from the resting muscle under greater initial passive stretch and tension, we find (fig. 5, C, D) to be less steep, the ascent duration longer, and therefore the summation-number larger, and the ascent-plateau turn more gradual and open, than when the initial passive stretch and tension are less. The plateau tension developed under the same stimulus intensity to motor nerve was often actually greater under higher initial passive stretch and tension than under lower. But with increasing passive stretch a limit is soon reached beyond which further increase of stretch proves unfavourable to the development of contraction tension. There is an optimal degree of initial passive stretch and tension favouring the development of contractile tension (Blix (4), Doi (5), Hartree and Hill (6)). Tibialis anticus shows (fig. 5, A, B) itself affected by initial passive stretch in a manner and degree closely similar to semitendinosus.

The influence of initial stretch and tension on the form of the isometric twitch in these mammalian muscles resembles that ascertained for it in excised frog-muscle by various observers. In the three muscles examined increase of the initial stretch within limits increases the duration of the crest, prolongs the relaxation-period, and the maximal tension developed by the twitch is often considerably increased.

We find that these circumstances, namely stimulus frequency and intensity, and passive stretch of the resting muscle, which thus influence the mn. tetanus,

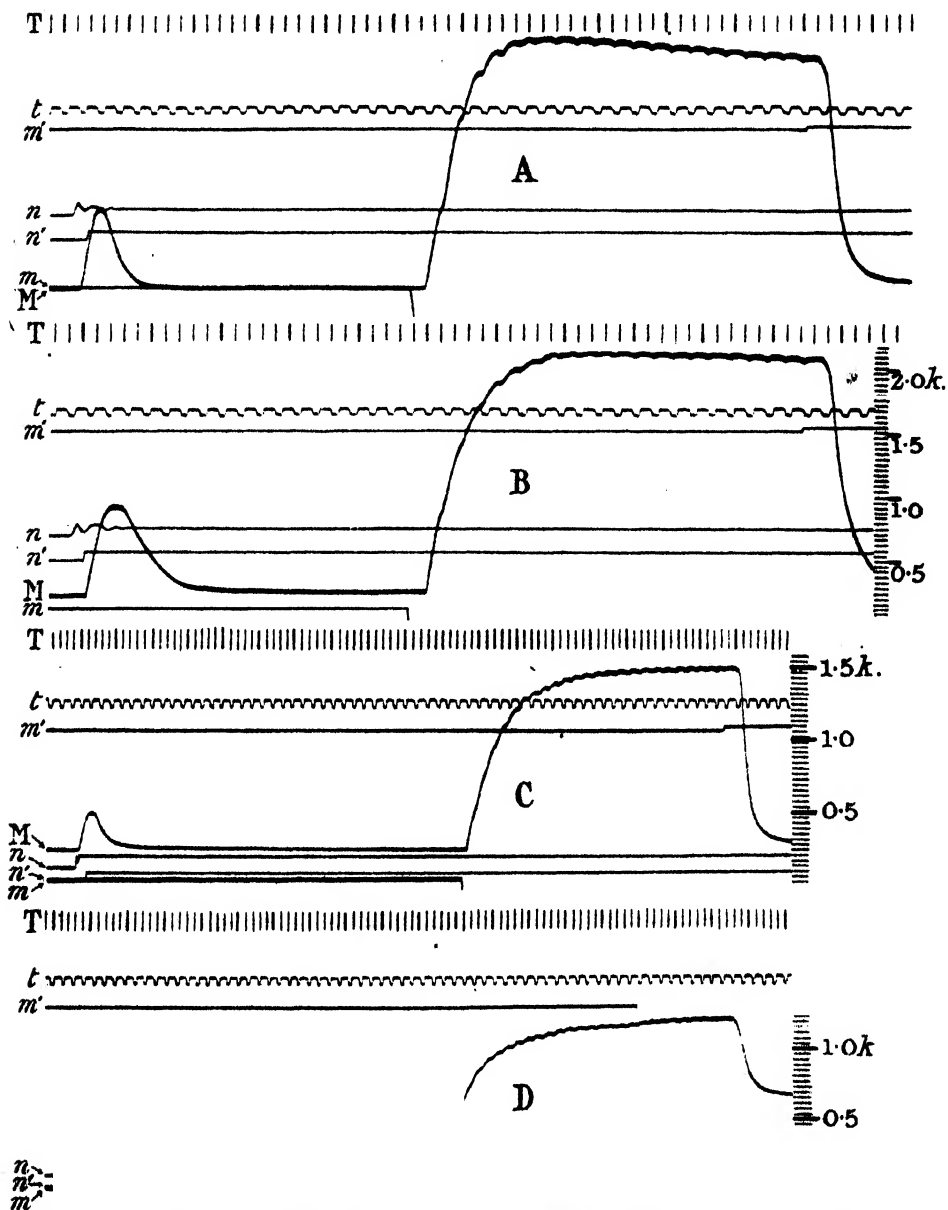


FIG. 5.—A and B. *Tibialis anticus*: In B initial passive stretch increasing initial tension of muscle by 0.3 lb., motor nerve stim'd., coil 18 cm. in both A and B. Stim. freq. 39 p. sec. T, 0.02 sec. Myograph magnifying tendon movement 60 times. Tension calibration on B, C and D. *Semitendinosus*: In C initial passive stretch giving initial tension of 0.4 lb. to muscle. In D passive stretch increased. Motor nerve stim'd., coil 12.75 cm., i.e., maximal in both. Stim. freq. 39 p. sec. T, 0.02 sec. Myograph magnification of tendon movement 38 times. Tension scale calibrations at side.

affect in a like sense the reflex contraction. The greater the frequency of the stimulus, the steeper, under otherwise similar conditions, the ascent and the sharper the ascent-plateau turn of the isometrically recorded reflex tetanus.

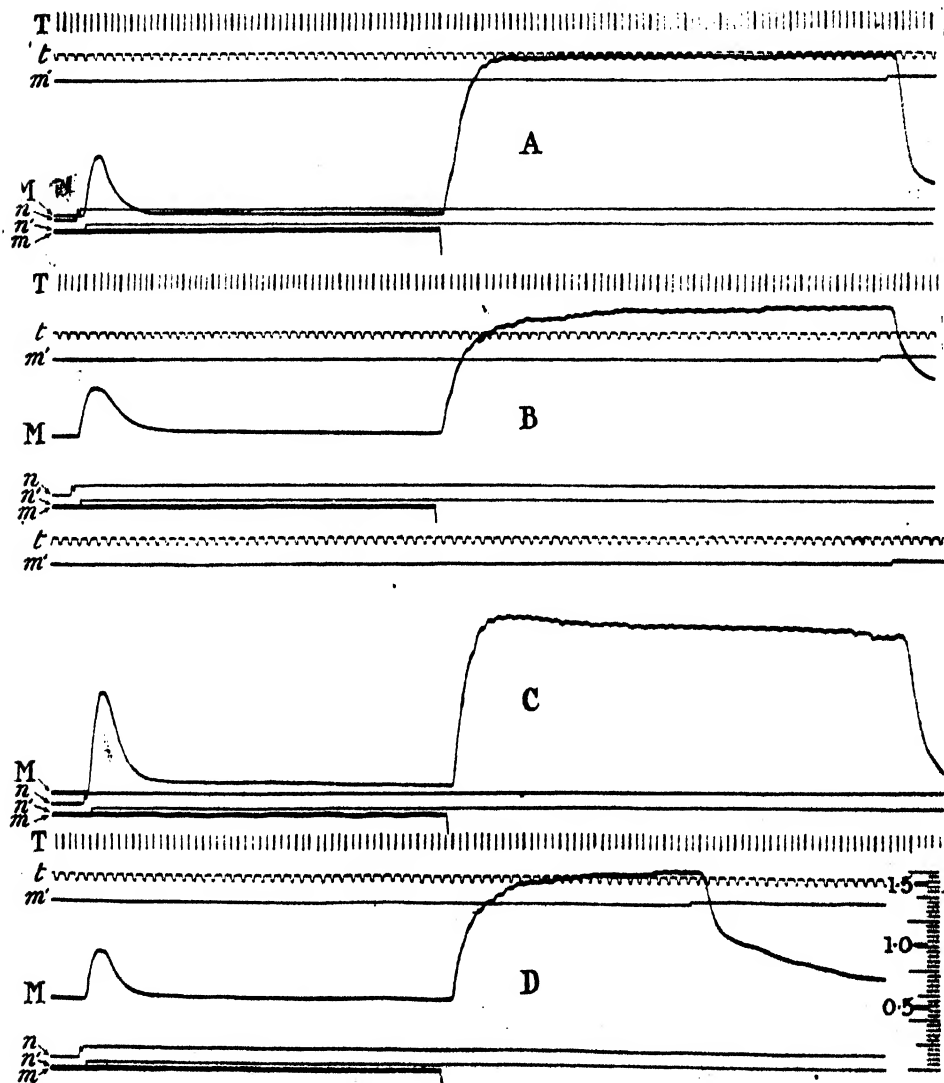


FIG. 6.—*Semitendinosus* reflexes; ipsilat. peron. popliteal nerve stim'd. in A and B coil 18 cm. In B initial passive stretch of muscle much the greater. C and D coil 16.8 cm. In D initial passive stretch of muscle much the greater; after-discharge more than in C. Stim. freq. 39 p. sec. in all. T, 0.02 sec. Calibration scale of tensions on D, applicable to all. Myograph magnification of tendon movement 38 times.

Again (fig. 9, B, D) the greater the intensity of the stimulus, *ceteris paribus*, the longer the ascent-time and the greater the number of ascent-steps in the reflex tetanus; and the sharpness of the ascent-plateau turn tends to be particularly marked with weak reflexes and weak stimuli. Greater initial passive stretch and tension of the resting muscle, within limits, other conditions being similar, augment in the reflex tetanus (fig. 6, *semitendinosus*), the ascent-time, and the number of steps to reach the plateau, and tend to make the ascent-plateau turn more gradual and open, and to give a horizontal or slightly rising plateau instead of a somewhat declining one (fig. 7, *tib. antic.*) Initial stretch of the

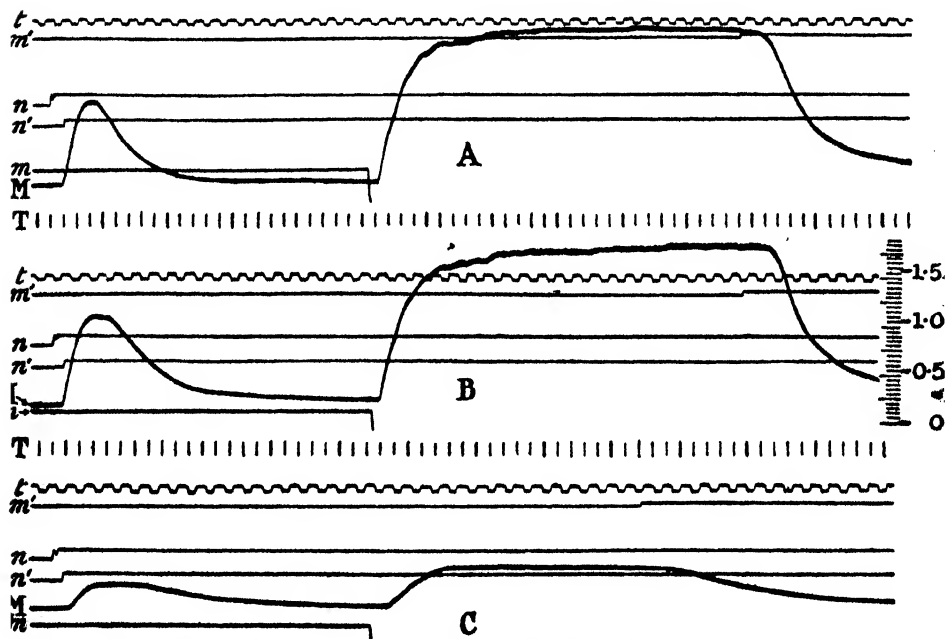


FIG. 7.—*Tibialis anticus* reflexes; ipsilat. popliteal nerve stim'd. In B, initial stretch about 4 mm. beyond length of muscle in A, giving 300 grms. greater initial tension. C, from smaller cat, initial passive stretch greater, giving more tension. Coil in all 13.8 cm., stim. freq. 38 p. sec. T, 0.02 sec. Myograph magnified tendon movement 60 times. Tension calibration on B.

resting muscle affects also the single shock reflex contraction in the sense of prolonging its crest and prolonging its period of relaxation. It prolongs also the terminal subsidence of the reflex tetanus. It also, within limits, causes the tension developed at the tetanic plateau to be greater; but with these two flexor-reflexes the limit to the initial stretch which increases the plateau tension is, as with the mn. tetani themselves, relatively soon overstepped.

The conditions mentioned as influencing the mn. tetani are thus found to exert in a similar sense their effect also on the reflex tetani: in the reflex contractions, therefore, distinction has to be made between the features of peripheral nature and those which are purely reflex. Thus a character in these reflex tetani even of quite short duration is that under stimulus frequency too low for complete fusion of the tensile waves the crest of the final contraction wave of the plateau is more prolonged than is that of a single-shock reflex just preceding the tetanus (*e.g.*, fig. 6). In view of the higher mechanical tension at plateau level than in the single-shock reflex's crest, this might hardly be expected. It is a feature which, though present in the reflexes, must be of peripheral nature. In the mn. tetani it is at least as pronounced as in the reflex (*e.g.*, fig. 5, A, C). It holds also as between the crest of the final tetanic-plateau wave and that of a preceding two-shock contraction (fig. 20, C). It is presumably connected with the change noted by W. Hartree and A. V. Hill (6), in the time-relations of the isometric twitch when it is repeated in building up a tetanus.

Further, both in mn. tetani and in reflex tetani, the tensile waves of contraction fuse earlier in the progress of tetani under higher passive stretch than under lower (fig. 5, C, D, and fig. 6, C, D).

For comparison of the reflex tetanus curve with that of the mn. tetanus, it would be desirable could all the influencing conditions be similar for both. To assure similarity of stimulus-frequency offers no difficulty. But as regards the stimulus-intensity there is no strict basis of equality, because the threshold value of stimulus obtaining for the reflex is not the same as that obtaining for the mn. tetanus, the latter being always the lower. There can, however, be taken for comparison reflex and mn. tetani of approximately similar plateau height, *i.e.*, developing similar plateau tension. When this is done, the reflex ascent-curve is in our experience very usually the shorter in duration, the steeper, and in its ascent-plateau turn the more abrupt (figs. 8 and 9). That these features have been lent to the reflex tetanus in our observations by the initial stretch of the muscle for the reflex tetanus having been consistently less than for the mn. tetanus is improbable, although owing to a certain amount of tonic self-adjustment of length in the reflex muscle it is difficult sometimes to adjust the tension of this latter.

A frequent difference between the reflex tetanus and the mn. tetanus is that the plateau in the former instead of maintaining itself parallel with the horizontal time axis tends to decline as it progresses (*e.g.*, fig. 9). This is so especially with weak reflexes, *e.g.*, with reflex tetani for which the

stimulus is relatively of low intensity. And this decline often sets in very early, *e.g.*, immediately succeeding the ascent-plateau turn.

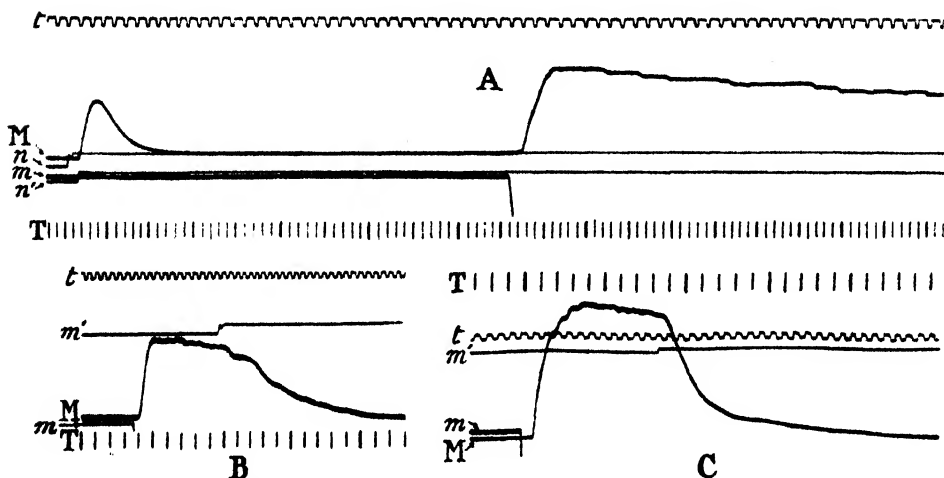


FIG. 8.—A. *Semitendinosus* reflex, ipsil.-peron. popliteal nerve stim'd., coil 14.2 cm., stim. freq. 38 p. sec. T. 0.02 sec. B. and C. *Tibialis anticus* reflex, ipsil.-popliteal nerve. In B, coil 14 cm. stim. freq. 50 p. sec. In C, coil 16 cm., stim. freq. 40 p. sec. T, 0.04 sec.

When examined in tetani made up of contraction waves incompletely fused, a frequent difference between the reflex ascent-curve and the mn. tetanus ascent-curve is found in the greater height in the former of the initial contraction steps relatively to the later ones (figs. 7 and 8). This difference is found so frequently that it may be regarded as characteristic. And it has occurred in instances where all difference between the reflex and the mn. preparation in regard to initial stretch of the resting muscle has certainly been excluded (figs. 9 and 10). To examine this further, we have made observations in which a single shock, similar to those composing the series used less than a second later for excitation of the tetanus, was taken to excite the single shock contraction either twitch or reflex (figs. 7, 9 and 10). With the reflex preparation a single shock reflex thus preceded at short interval the reflex tetanus composed of such single reflexes; and with the mn. preparation a twitch preceded the tetanus built up of such twitches. The single-shock in each case was obtained by unshorting for a suitably brief interval the derived electrode circuit of the secondary coil, and so similarly the tetanic series. The ratio between the height (tension) of the single-shock reflex and that of the reflex tetanus thus observed, was compared with the ratio

between the height (tension) of the twitch and of the mn. tetanus. Denoting these ratios  $ss/tet\ refl.$  and  $ss/tet\ mn.$  respectively,  $ss/tet\ refl.$  is found to be the greater and often much the greater.

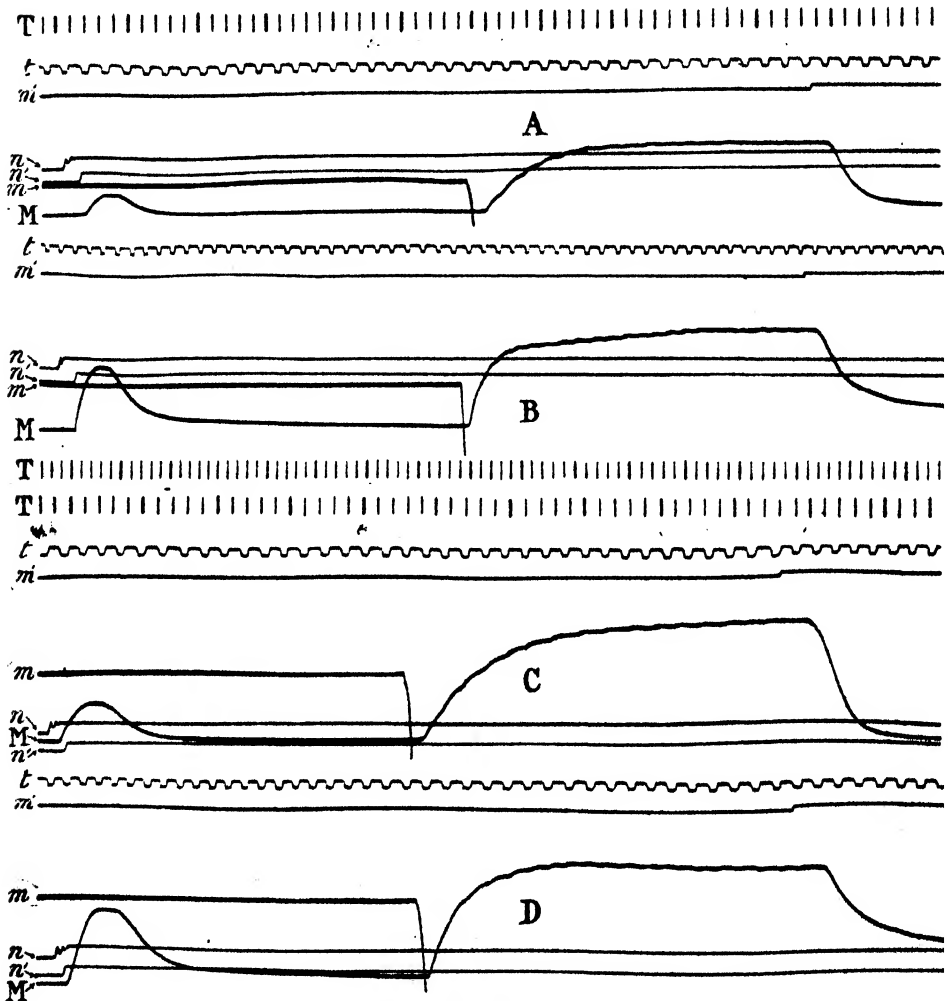


FIG. 9.—*Semitendinosus*, single shock/tetanus ratio. A. motor nerve stim'd., coil 16 cm. B. reflex, ipsil.-peron. popliteal nerve, coil 15.8 cm. C. motor nerve, coil 16 cm. (maximal); D. reflex, ipsil.-peron. popliteal nerve 15.5 cm. Stim. freq. 38 p. sec. T, 0.02 sec.

In our hands both ratios exhibit not inconsiderable variation, for our purpose a somewhat disappointing circumstance. They seem affected by the intensity of the stimulus, and also by the initial stretch (fig. 18). But these variations

are not sufficient to obscure the broad result that the ratio in the reflex is greater than in the mn. reaction. In the latter the ratio rarely exceeds 40 p.c.; in the former it is rarely so low as 40 per cent. We consider this

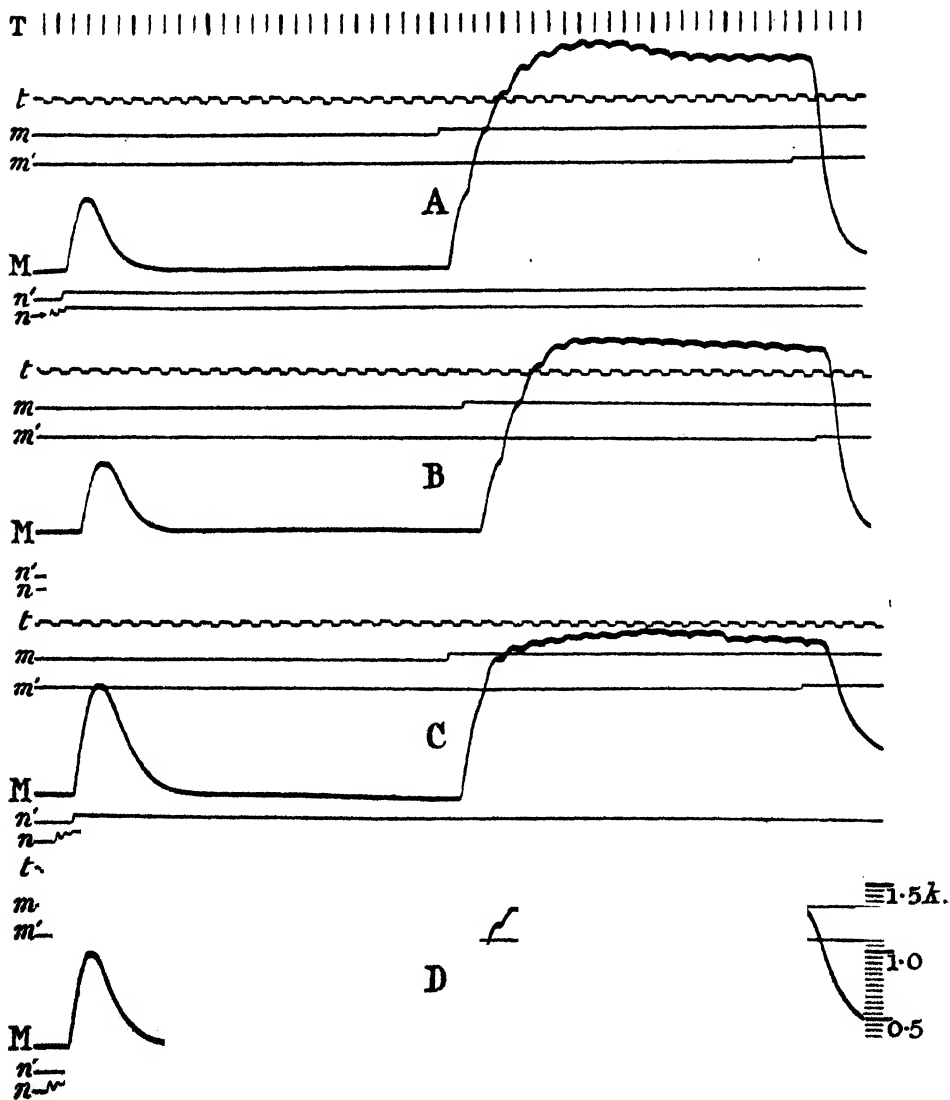


FIG. 10.—*Tibialis anticus*, single-shock/tetanus ratio. A and B, motor nerve stim'd., coil at 18.5 cm. in A, 21 cm. in B. C and D, reflexes, ipsil.-popliteal nerve, coil at 13.8 cm. in C, at 14.5 cm. in D. In C the initial passive tension (stretch) rather greater than in rest. Stim. freq. 39 p. sec. T, 0.02 sec. Myograph magnification of tendon movement 60 times; tension calibration on D for all.



difference characteristic as between the development of the reflex and the mn. tetanus respectively (fig. 11, A, B).

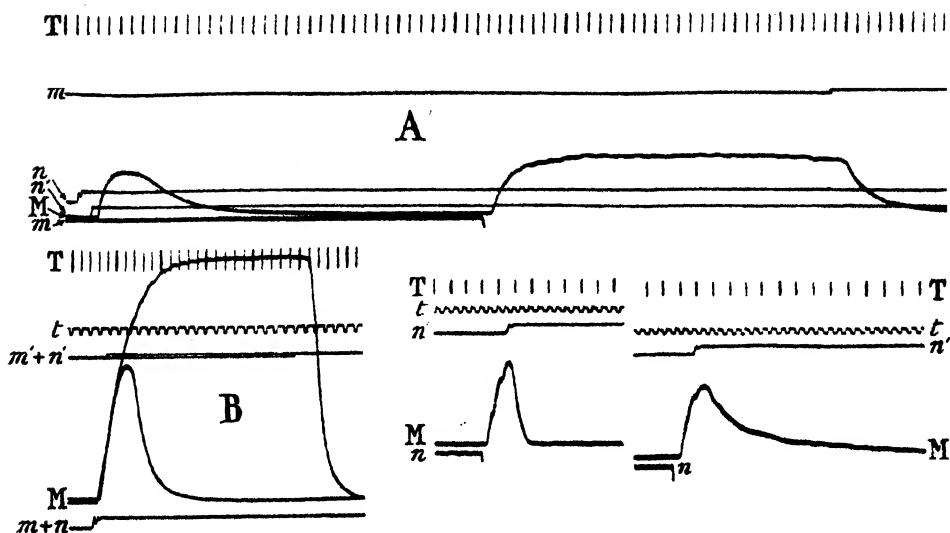


FIG. 11.—A and B. *Semitendinosus*. A, single shock/tetanus ratio, reflex, ipsil. peron. popliteal nerve stim'd., coil 15.8 cm., stim. freq. 38 per sec. T, 0.02 sec. B, motor nerve stim'd., two-shock reaction compared with short tetanus, coil 20.2 cm., stim. freq. 50 per sec. T, 0.02 sec. C. *Tibialis anticus*: (i) motor nerve stim'd., three-shock tetanus, coil 20 cm. (ii) three-shock reflex, coil 14 cm. Stim. freq. 50 p. sec. T. 0.04 sec.

When we seek meanings that the mn. tetanus curve conveys, one is the following. So long as the serial stimulus consists of individual stimuli of similar intensity throughout, the isometric record gives the additive curve of development of contractile tension for a definite number of the fibres of the muscle, and whether that number be larger as in strong tetani or smaller as in weaker tetani, that number, whatever it be, remains unchanged from beginning to end of the reaction. The very horizontality of the plateau level above the abscissa base-line guarantees that interpretation for that period of the tetanus, and its acceptance for the ascent period also can hardly be challenged. No evidence of an *addition latente* has been detected in the mn. tetani of the vertebrate skeletal nerve-muscle preparation under ordinary rates of stimulation with equable stimuli. That it can appear under abnormal conditions and special frequencies of stimuli inducing impulse interference does not apply to preparations in which, as in ours, circulation and temperature were maintained, no drug was used, and the rates of stimulus frequency were

far removed from such as would under such circumstances cause impulse interference in the warm-blooded preparation. We conclude, therefore, that the mn. tetanus provides for us the curve which exhibits the tension development of the tetanic contraction of the muscle under a given frequency of stimulus when the number of muscle-fibres, and inferentially of motor-nerve fibres, involved in the reaction remains one and the same throughout the individual tetanic reaction from its beginning to its end. We are considering here reactions rarely exceeding some two seconds' duration, from which, therefore, fatigue in any ordinary sense of the term can be excluded.

Broadly taken, the striking general resemblance between these spinal flexor reflex tetani and the mn. tetani of their respective muscles is so close as to suggest that the inference just drawn from the mn. tetanus curve holds also for the reflex tetani. And this the more since, as shown above, certain variations in the contraction curves both of the reflex and mn. reactions, variations which, inasmuch as they are of peripheral source, attach to both those reactions, are on examination found noticeably influencing both reactions in the same sense.

There exists, however, some discrepancy between the form of these reflex tetani and of the mn. tetani; thus, the relatively steep ascent and relatively abrupt ascent-plateau turn of the reflex curve as compared with the curve of the mn. tetanus. Taking the reflex curve at its face value, an inference from this difference would be that the number of motoneurones activated by the reflex begins to diminish progressively from its very outset.

When it was said above that the first steps of the reflex ascent are high relatively to those of the latter steps as compared with the mn. tetanus, the comparison made was between reflex and mn. tetani of comparable plateau height; and the question arises whether as compared with the ascents of mn. tetani the first steps of the reflex tetanus ascent are of exaggerated height or its later steps are of excessive lowness, *i.e.*, exhibit excessive deficit of contribution to the height of the ascent. From examination of the records we think that the reflex ascent includes both these features. Taking the latter first: that in the successive increments of tension given by the later steps of the reflex ascent there is a decline which is excessive as compared with those of the mn. tetani harmonizes with the falling slope of plateau which the reflex tetanus often early shows especially under weak stimuli. This feature might indicate that as the reflex proceeds some of the motoneurones which it has activated slip, so to say, from its grasp, and especially under weak stimulation do so early. The relative failure of increment to the ascent in its topmost steps may attach to early onset of failure on the part of the reflex to

maintain the discharge of some of the total number of motoneurones it initially activated. But the dwarfing of height of the later steps of the reflex ascent is also unmistakable in many reflex tetani in which the plateau does not early decline. So that the above supposition of premature failure of the reflex strength is not in a number of instances adequate. This point will be returned to after re-consideration of the character of the first steps of the ascent.

As to these and their disproportionate size, it must be remembered that after-discharge, though not so extensive in these spinal flexor reflexes as in the decerebrate extensor reflex, is yet, in our experience, never wanting altogether. It is a process which is obviously central though ensuant upon the external stimulus, the central effect of which it prolongs in the forms of motoneurone discharge for a short though variable time. The after-discharge in these reflexes, though never absent, never in our records produces any regular contraction rhythm, despite the recording method being able to reproduce any rhythm which the muscle itself is not too sluggish to show, provided only that it is not grossly asynchronous in the aggregate motoneurone group involved.

The after-discharge must mean some after-persistence of central reaction to the centripetal impulses received by the centre from the stimulated afferent nerve; and that persistence must, as expressed by the efferent motor fibre, be impulse-discharge of repetitive character. The observations of Forbes (7) (19) and others (20) indicate that the frequency of rate of such a repetitive process is high, too high for corresponding mechanical rhythm in the muscle. In conformity with that evidence is the absence of mechanical rhythm in the contraction evidencing this reflex after-discharge. A question of importance for us here is how early during the reflex tetanus does this central after-action begin. We find it develop quite early. Not only is it a "terminal phenomenon" (T. Graham Brown (12)) of reflexes which have been maintained for 2 or 3 seconds, but it occurs also and quite markedly when the reflex is abbreviated by curtailing the period of application of the external stimulus to, *e.g.*, less than 0.1 second. It has begun, therefore, even during the ascent period of the reflex tetanus. It is fully evident in reflexes excited by so few as three and two stimuli to the afferent nerve (fig. 11, C). Further, in conformity with this, the reflex contraction excited by a single shock often shows a course of subsidence indistinguishable from that of after-discharge (8). In such a case it commences within the period of a single-shock reflex, that is, it complicates the first contraction step of the reflex tetanus, which in virtue

of its repetitive frequency it will heighten. This would account for the relatively excessive size of the earliest contraction step of the reflex tetanus ascent. Further, characteristic of reflex after-discharge is the length of its duration in some of the motoneurons it affects. With rates of serial stimuli, such as we have used commonly for the reflex tetani, *e.g.*, 35 to 50 per second, the centripetal impulses from the second stimulus will, on reaching the centre, find some of those motoneurons which they, being of like intensity to the first, should be competent to excite, still occupied with the high-frequency repetitive reaction, *i.e.*, the after-discharge, excited by their predecessors of the first stimulus. These motoneurons still engaged in after-discharge they will be unable to excite; a point recently insisted on by Forbes, Cobb and Cattell (9). They will, therefore, be able to excite only a fractional number of that total number of motoneurons which they excited at first. The second and ensuing reflex contraction waves of the reflex tetanus will, as compared with the first reflex contraction wave, contribute, therefore, disproportionately less to the contraction ascent than do the second and ensuing waves as compared with the first in the mn. tetanus where there is no after-action. Inspection of records of the reflex augmentation due to an incremental stimulus (fig. 2, B, C) shows that the disparity between the height of the first and later steps of the ascent is less in the incremental ascent than in the original ascent. This difficulty for the explanation just offered on the view that the incremental contraction is obtained by accession of fresh motoneurons is, however, lessened by the fact that the mn. tetanus also in the steps of its incremental ascent shows similarly less disparity in height than do the steps of its original ascent. The lessening of the disparity seems, therefore, of peripheral source and would affect the reflex as well as the mn. result. It may be related to the small-stepped re-ascent of an interrupted mn. tetanus (1) (fig. 3, C).

The rhythm of the external stimulus even up to 60 per second and more gives tensile waves of corresponding rate in the tetanic contraction of these reflexes. That this rhythm is evident despite the after-discharge is consonant with the after-discharge, in these reflexes, never maintaining the plateau level, that is, if a deduction be made after the end of the stimulus of a time equal to the reflex latency of commencement. There are, therefore, in these reflexes some of the motoneurons in which, even in the fully developed tetanus, after-discharge, if present, ceases very rapidly indeed. But terminal after-discharge in these reflexes increases markedly with increase of intensity of the external stimulus.

We conclude that the features of the reflex isometric myogram, as sampled by the two muscles examined, a knee flexor and an ankle flexor, signify that in this type of reflex, *i.e.*, spinal ipsilateral flexion reflex, (1) the reflex reaction to a continued repetitive stimulus of unchanged frequency and intensity engages initially a number, greater or smaller according as the stimulus is stronger or weaker, of the totality of the motoneurones passing to the muscle, which initial number is from the very outset the full quota of the totality of the muscle's motoneurones which the reflex will, under continuance of that stimulus, engage; (2) that that initial number in the further course of the reflex, though not exceeded, appears in many cases to be maintained, but in some cases, especially under weak stimuli or in weak reflexes, progressively diminishes although the external stimulus continues unabated; (3) that these features attach to these reflexes not only when the spinal transection has been made shortly before observation, but also when the transection has been a couple of weeks earlier; and occurs under skin stimulation as well as under direct stimulation of the afferent nerve; (4) that in this type of reflex the intensity of the reflex, other things being equal, is conditioned by the intensity of the stimulus, the stimulus if stronger activating a larger number of the motoneurones than if weaker; mere lengthening of the duration of the stimulus does not augment the reflex contraction, this latter on the contrary tending to wane despite unaltered continuance of the stimulus. In other words, of the external stimulus' two factors, intensity and duration, the former is in this type of reflex the sole arbiter of the intensity developed by the reflex contraction.

#### IV.—*Crossed Extensor Reflex (Decerebrate).*

This type-reflex we have sampled in the quadriceps extensor of the knee. Its isometric myogram differs greatly from that of the foregoing reflexes. We have studied it in decerebrate preparations made by ablation of the whole brain anterior to the posterior colliculi. Adhering for descriptive convenience to the terms "ascent" and "plateau" these show in our records features as follows:—

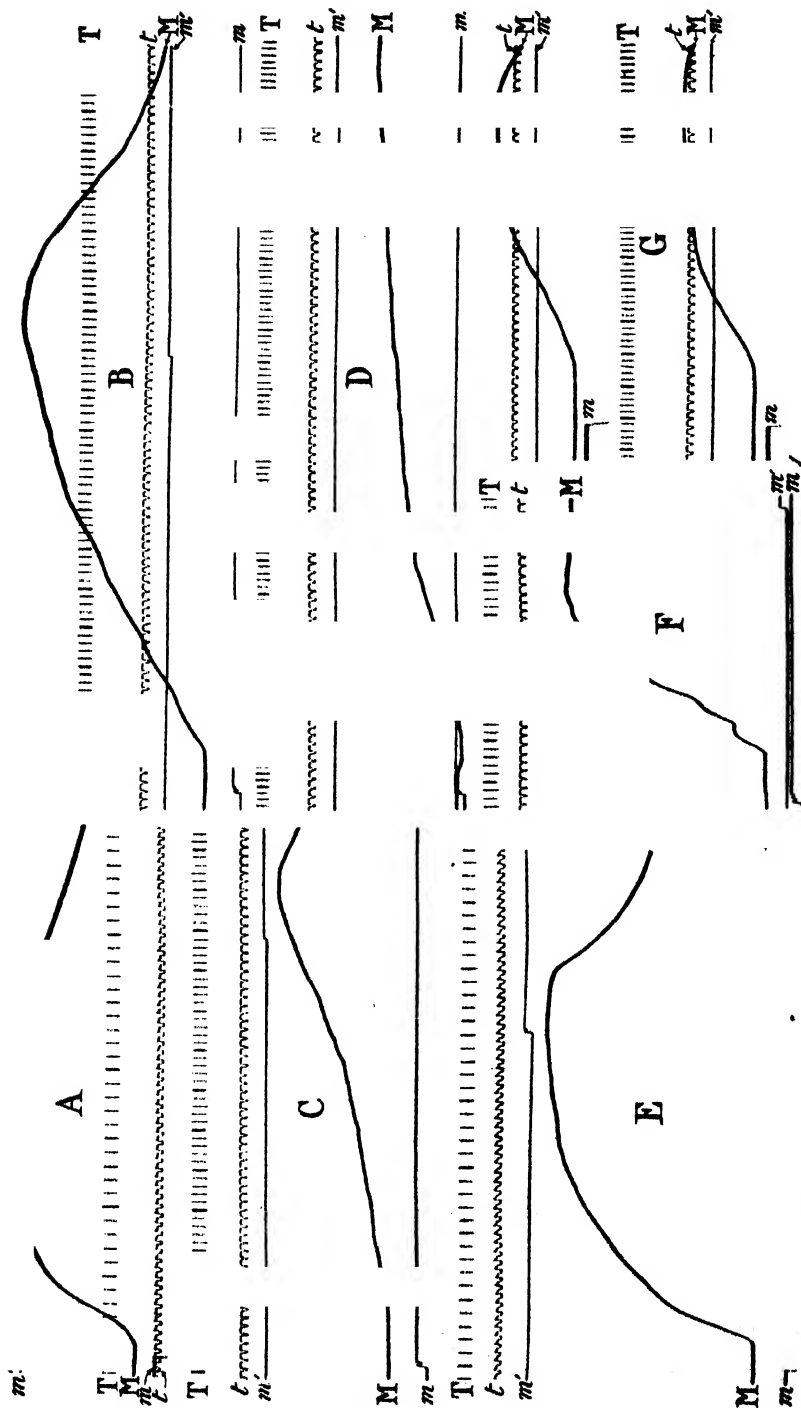
(1) With single-shock series of frequency 30 per second and upwards, the ascent and plateau unlike those of the previous reflexes show either no trace of contraction undulations corresponding with the stimulus-frequency, or mere traces at the lower of those frequencies (1). No "summation-number" is therefore directly legible by inspection of the ascent of the reflex.

(2) But the ascent-duration, *i.e.*, the period between beginning of the con-

traction and the attainment of the plateau, is, under the same frequency of stimuli, longer for this reflex than for the flexion reflexes (preceding section). It is also longer than for the mn. tetanus of quadriceps extensor itself. Often this excess of duration in the reflex is very great indeed (fig. 12, B, C, D ; fig. 13), *e.g.*, the reflex ascent may have ten or even twenty times the duration of that of the mn. tetanus.

(3) The form of curve of the ascent differs greatly from that of the mn. tetanus, and from that of the flexion-reflexes (preceding section). It is commonly sigmoid (fig. 12, A, G), being less steep in some part of its early course than later, and then after attaining its steepest gradient becoming progressively less steep as it approaches finally the plateau. But the ascent as compared from experiment to experiment presents much latitude of departure from the typical sigmoid form. An infrequent sub-variety is one where the ascent opens with a somewhat quick gradient, on which follows a slower or even horizontal course, to be succeeded by a further less gradual rise which climbs then with gradually diminishing steepness into the plateau. The ascent in such instances is made in two, or there may be more, slow steps (fig. 12, F). Another sub-variety of the ascent is one presenting a long slow approximately rectilinear gradient (fig. 12, D) which, subject to small irregularities, steadily climbs even throughout 3 to 4 seconds, and merges finally into a horizontal plateau only after withdrawal of the stimulus itself. The most common form of the ascent is, however, as said above, a smooth sigmoid curve steepest in gradient somewhere about half way between base-line and plateau-level. In many cases the distinction between top of ascent and beginning of plateau is arbitrary because the plateau itself climbs somewhat, and the ascent-plateau corner has a very open angle.

On comparing the features of this reflex curve of ascent with the features of the ascent-curve of the mn. tetani of the extensor muscle itself (fig. 13), wide dissimilarity is obvious between the two. The latter are not much different from those of the flexor muscles as described above. It is true that in our experience the summation-number and ascent-period are rather longer for this extensor muscle than for the two flexor muscles examined under the same frequency of serial stimulation. And the plateau of the mn. tetanus of the extensor has, in our experience, more frequently and more prominently a tendency to climb after it has become approximately rectilinear. These characters of departure from full agreement with the flexor muscles are, it will be noticed, in the same general direction as some of the features distinguishing the extensor reflex. To judge how far the mn. mechanism of the



12.—*Quadriceps extensor reflexes* selected from various separate experiments, contralat. peron. popliteal nerve stim'd. A, coil 13.8 cm., freq. 39 p. sec. B, coil 15.5 cm., freq. 49 p. sec. C, coil 15.5 cm., freq. 49 p. sec. D, coil 16.5 cm., freq. 38 p. sec. (for early relaxation, cf. T. Graham Brown (*gastrocnemius*), 'J. of Exp. Physiol.', vol. 255). E, coil 15 cm., freq. 39 p. sec. F, coil 17.5 cm., freq. 49 p. sec. G, *Vastus externus* and *crureus*, upper curve, coil 13.8 cm.; lower, coil 14.5 cm. Stim. freq. 36 p. sec. T, 0.04 sec. in A and E, 0.02 sec. in rest.

extensor contraction might account for the form of the extensor's reflex curve we have, therefore, sought what conditions accentuate them in the extensor's mn. tetanus itself.

• As with the mn. tetani of the flexor muscles so also with those of this extensor

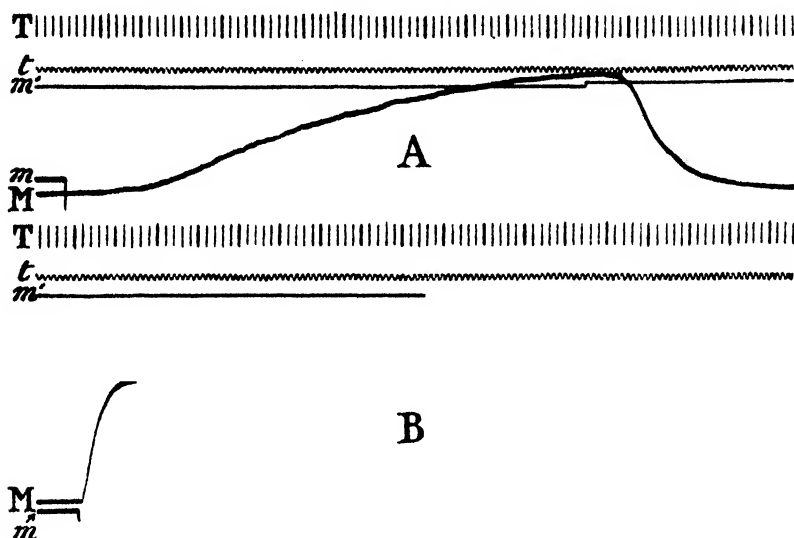


FIG. 13.—*Quad. extensor.* A, reflex contralat. peron. popliteal nerve stim., coil 14.5 cm. freq. 38 p. sec. B, motor nerve stim'd., coil, 22 cm., freq. 38 p. sec. T, 0.04 sec.

muscle, conditions which tend to prolong the ascent and open out the ascent plateau corner are : (1) lesser frequency of the serial stimulus rate (fig. 3, A), (2) greater intensity of the serial stimuli, (3) greater passive stretch or tension of the muscle when the tetanus is provoked (fig. 14, B; fig. 18). Of these conditions the last named exerts in the case of this extensor muscle a greater and more complex influence than—in our experience—in the case of the two flexor muscles. It affects greatly the whole value of the mechanical response as examined by the isometric method. Under increased passive initial stretch of the muscle a motor-nerve stimulus of the same intensity and frequency as applied, just previously or just subsequently with the muscle under less passive stretch, will evoke a tetanus developing double or more the tension of the tetanus provoked when the passive stretch is less (fig. 14, B). And the range of initial passive stretch, as estimated by the passive tension that it produces, through which the augmenting effect is evident, is greater than with either of the two flexor muscles. It seemed in our observations that increase of the initial passive stretch of the muscle sometimes actually lowered the threshold value of stimulus for the mn. twitch and tetanus.



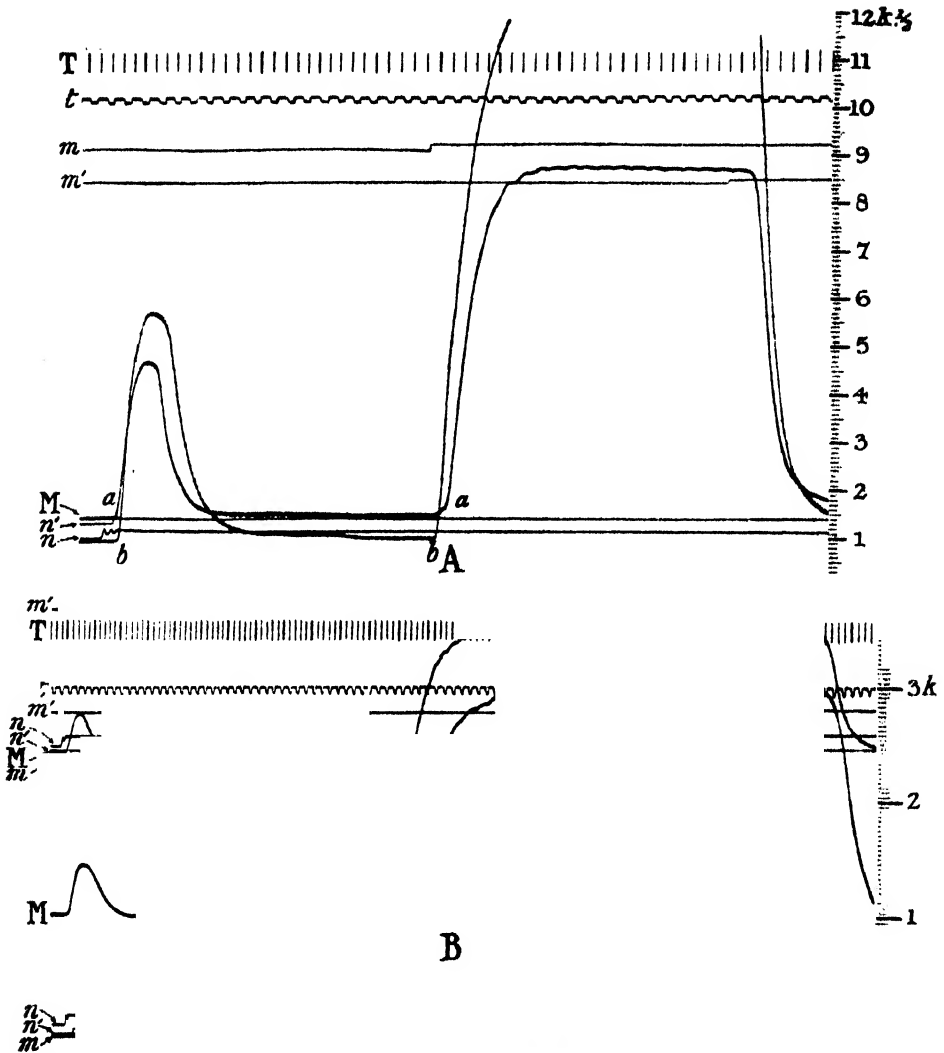


FIG. 14.—A, *Vasto crureus*. Motor nerve, stim'd., approximately maximal, coil 19 cm., freq. 38 p. sec. For curve *b* initial stretch of muscle increased by 6 mm. Total length of muscle 93 mm.: magnification of tendon movement by myograph 162; tension calibration in  $\frac{1}{2}$  kgms. at side; weight of cat 2850 grms. T, 0.02 sec.; small vibs. in myogr.,  $1/250$  sec. B, *Vasto crureus*. Motor nerve stim'd., submaximal, coil 24.2 cm., freq. 38 p. sec. Calibration of tension given in kgms.; zero of tension is the level of the signal line *m* (unshorting for tetanic stim.) for each observation. Under the greater initial stretch the plateau tension level is just short of 3 kgms.; under the less initial stretch (upper myogram) plateau tension falls short of 1 kgm.

*Examples :*

Obs.	Second coil.	Initial stretch in terms of passive tension.	Twitch tension.	Tetanic tension.	Initial and tetanic tension.	Steps in ascent.
(1)	cm. 26.8	gms. 200	230	900	1160	9
(2)	26.8	900	400	1800	2700	15
(3)	24.2	150	250	900	1050	8
(4)	24.2	1050	400	1800	2850	13

The high initial tensions correspond with initial stretches of about 4-6 mm.

The influence of passive initial stretch on this muscle is in our experience so marked that, although our examination of it has been simply precautionary, to avoid imputing to reflex mechanism effects really attributable to motor-nerve-muscle constituents, some further points may be mentioned. (1) It occurs with submaximal as well as with maximal contractions ; (2) it occurs with all of the four components of quadriceps taken separately, namely, crureus, vastus externus, vastus internus, and rectus femoris ; (3) the passive stretches of the muscle which produce it need not cause elongations of the muscle which amount to any high percentage of the length of the muscle ; e.g., 3 p.c. or 4 p.c. But these degrees of passive lengthening of the muscle correspond with considerable change in the angular posture of the knee-joint.

Hip flexed to 90° as in observations.	Length of quadriceps muscle from origin to insertion.
Knee fully flexed .. .. .	140 mm.
Knee flexed to 90° .. .. .	133 mm.
Knee fully extended .. .. .	125 mm.

Wt. of cat 2235 grms.

The favourable effect of passive stretch seems to diminish rapidly when the degree of stretch exceeds the amount corresponding with that given by maximal flexion of knee accompanying full extension of the hip. (4) The augmentation of the tensile response of the tetanus can be produced by passive initial stretches of the muscle which do not cause much increase in the passive initial tension of the muscle.

In the parallel-fibred sartorius muscle of the frog the influence of initial length of the resting muscle upon its development of tension in contraction

was studied by Blix (4), and has been recently with further results of capital importance by C. L. Evans and A. V. Hill (10), Doi (5), W. Hartree and A. V. Hill (6), and E. D. Adrian (11). Conditions attaching to our experiments render these latter unsuited for strict analysis of the effect of initial stretch on the form of the twitch and tetanus curves. The quadriceps and its component muscular masses are made up of muscle-fibre chains of extremely complex arrangement and of greatly different total length. Further, for their comparison with reflex contractions many of our mn. tetani have been submaximal and the distribution of the fibres employed in a submaximal tetanus must be largely matter of conjecture. But as we are not aware that mammalian skeletal muscle *in situ* has previously been laid under contribution for isometric myographic observations of this kind, we may add that it would seem from our experience that the favouring effect of increase of initial stretch on the development of active contraction-tension, although clearly demonstrable with all the three cat muscles, semitendinosus, tibialis anticus and quadriceps extensor examined by us, is considerably most so in the last named, the extensor of the knee.

It may be noted incidentally that the values of the maximal tetanic tensions developed in the quadriceps extensor have been a matter of some surprise to us. Thus, part of the vasto crureus muscle of a small cat weighing 2850 gms. developed in its tetanus, presumably maximal, a tetanic plateau tension of 6.6 kgms. If this formed about one half of the total quadriceps extensor, a maximal tetanus of the entire quadriceps would have developed a tension of 13 kgms. pull, and this with a stimulus frequency of 38 a second, which would hardly give the fullest maximal development of tetanic pull.

It was shown above that in the case of the spinal flexor reflexes the three factors: (1) stimulus frequency, (2) stimulus intensity, (3) passive stretch of muscle, which influence the mn. contraction, affect correspondingly the reflex contractions of those muscles in their spinal reflex. With the extensor muscle in its decerebrate crossed reflex the result is somewhat different, despite the circumstance that these factors affect the mn. contraction of the extensor muscle in the same sense as they do the contractions of the two flexors. (1) Increase of stimulus-frequency in the extensor reflex shortens the latent period, hastens the ascent and increases the steepness of the ascent-gradient. It acts, therefore, in so far, in the same sense as it does in the mn. tetanus and in the flexor reflexes. But the dissimilarity of the extensor's reflex ascent from that of the mn. tetanus and of the flexor reflexes is too great for these effects of stimulus-frequency upon it to come within strict comparison for

the two. Nor can, in our experience, any adjustment of mere stimulus-frequency bring these cases into line.

(2) As to the influence on the extensor reflex of stimulus intensity (fig. 15) increase of this latter shortens the latent period, develops a steeper and higher ascent and gives attainment of a greater plateau tension. These results are, it is true, in general direction like those it exerts on the mn. tetanus and

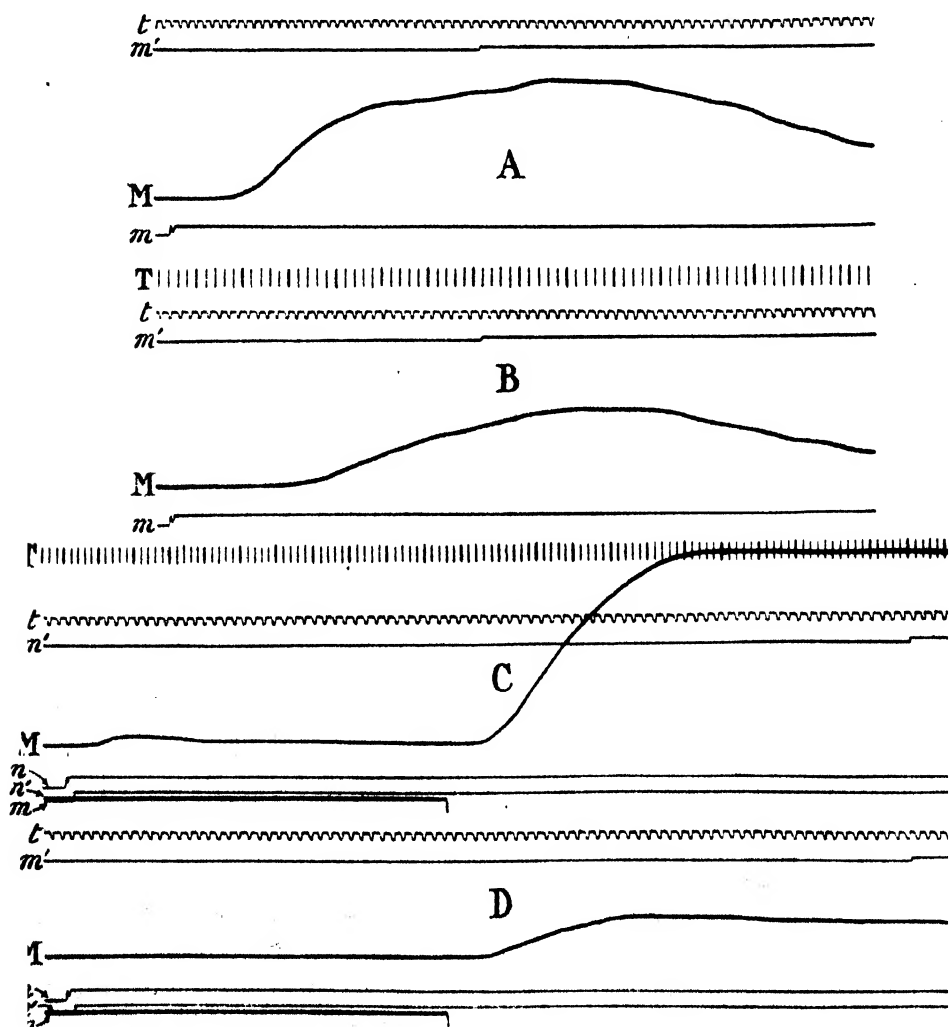


FIG. 15.—*Vasto crureus* reflexes, from contralat. peron. popliteal nerve. A, coil 15 cm. B, coil 15.6 cm., stim. freq. 50 p. sec., threshold 17 cm., consecutive obs. T, 0.02 sec. C and D. *Crureus*, s.s. followed by tet. series, in C coil at 14.5 cm., in D at 15.5 cm.; threshold 16.5 cm.; consecutive obs. Stim. freq. 38 p. sec. T, 0.02 sec.

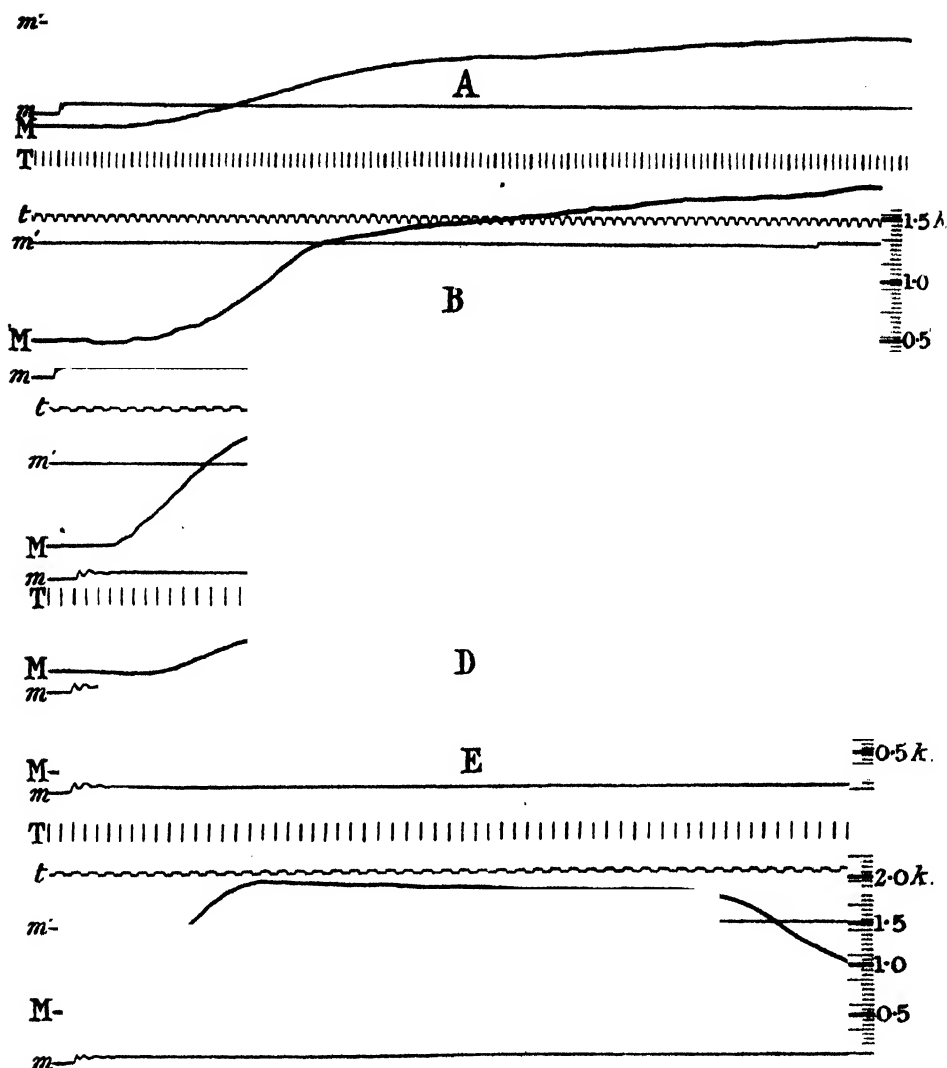


FIG. 16 (1).—*Quad. ext. reflexes, contralat. peron. popl. nerve.* A and B, consecutive obs.: coil at 36 p. sec. In B, initial stretch of muscle was increased by 5 mm. of muscle length, raising init. tens. T, 0.02 sec. Myograph magnifies tendon movement  $\times 38$ . C D E F. *Rectus fem.* consecutive obs.; coil at 13.8 cm., freq. 38 p. sec. In D and E initial stretch of muscle was same; in C was 4 mm. greater and in F 8 mm. greater than in C and D. The length of rectus to tibial insertion = 125 mm. with hip flexed to rt. angle and knee fully extended, = 133 mm. with knee flexed to rt. angle. T, 0.02 sec. Lever magnifies tendon movement  $\times 68$ . Calibration tension scale at B applies to A also; those at E and F apply to C and D also:  $m'$  is not repeated for D and E, but in the originals it fully corresponded with  $m'$  in C and F.

on the flexor reflexes ; but the form of the extensor-reflex remains throughout too discrepant from the others for immediate comparison, and adjustment of stimulus-intensity fails to reconcile them.

(3) So also with initial stretch of the resting muscle. This greatly augments the contraction tension obtainable in the reflex response (fig. 16 (1) and (2)), if the stretch be within the limits found favourable for it in the mn. reaction ;

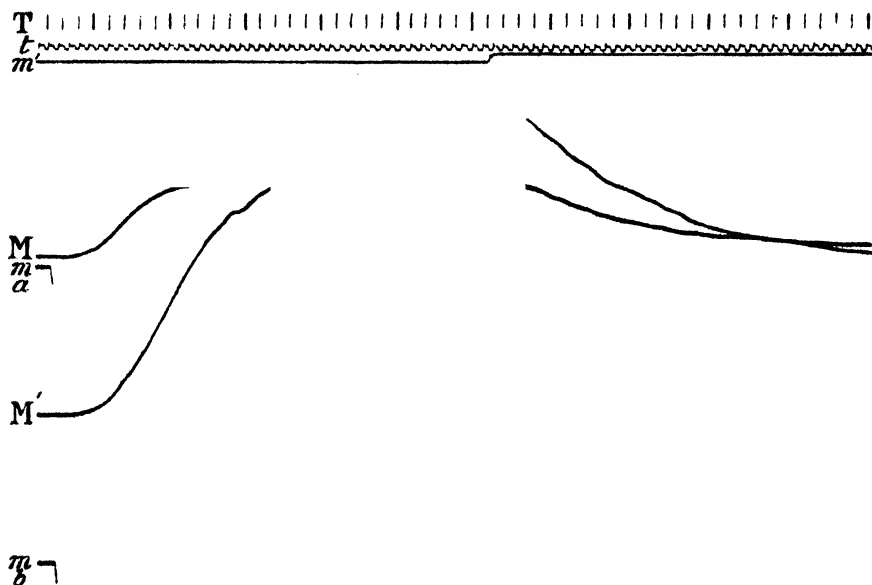


Fig. 16 (2).—*Vasto crureus*, reflex, contralat. peron. poplit. nerve : M and M' consecutive observations ; coil at 15.2 cm. for both ; stim. freq. 38 p. sec. For M' initial passive stretch of muscle was increased to 9 mm. more elongation of the muscle than in M, raising initial resting tension from 70 grms. up to 550 grms. Max. tension developed by reflex M < 400 grms. ; max. tension developed by reflex M' about 2000 grms., and much more after discharge. Myograph multiplies tendon movement 60 times. T, 0.04 sec. m' applies to both the stimulations. The vertical distances between M and Ma, and between M' and Mb represent the respective initial tensions.

and as with this latter initial passive stretch becomes unfavourable when that limit is exceeded. Whether in the reflex the influence of this factor is accentuated beyond its degree in mn. reactions is difficult to judge ; it certainly seems to be no less. Its favourable effect closely resembles that of an increase of stimulus intensity (fig. 17, A, B ; fig. 19, A, B) ; the latent period is shortened, the ascent is steeper and higher, the plateau-tension greater. It often renders a seemingly subliminal stimulus sub-maximal, thus in appearance lowering the threshold of the reflex. A single-shock that produced no

visible contraction in the slightly stretched muscle will on stretching the muscle a little further evoke an obvious reflex contraction (fig. 17, A, B). A

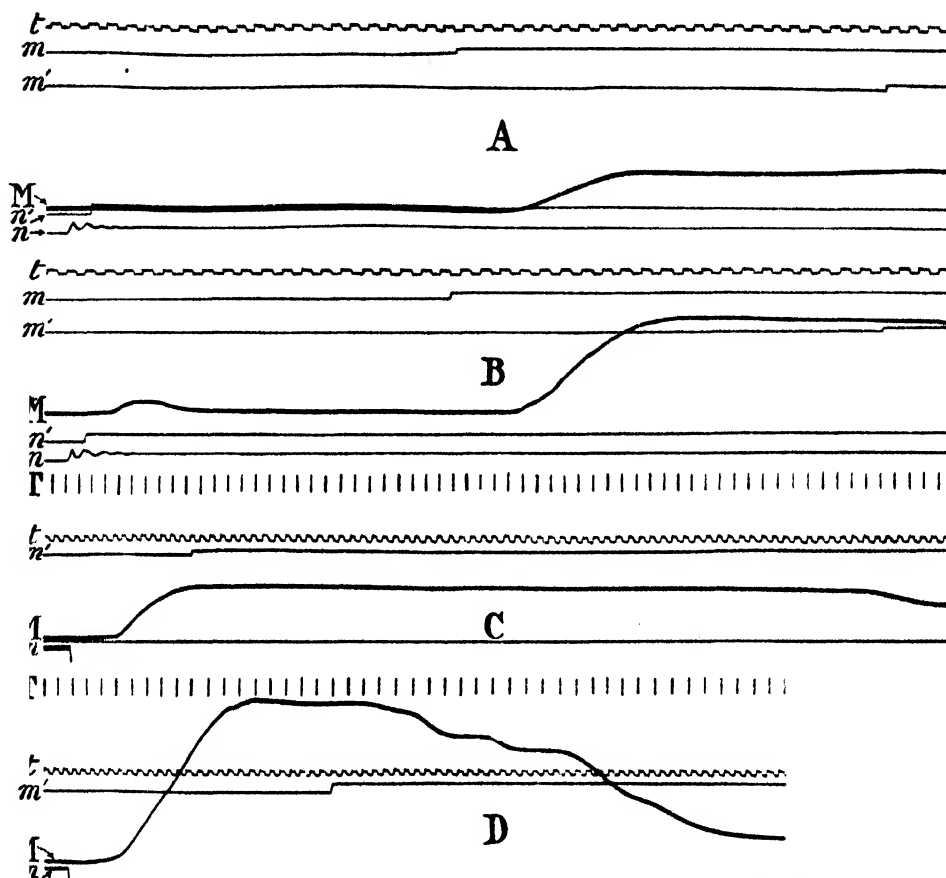


FIG. 17.—A and B, *Rectus fem.*, reflexes, contralat. peron. popl. nerve, coil 14 cm., freq. 38 p. sec., consecutive obs. In B, initial passive stretch was increased by 6 mm. raising init. tens. and this nearly trebled reflex plateau-tens. and also brought a response to s.s. stimulus preceding tet., which in A was absent. T, 0.02 sec. Myograph  $\times 68$ . Same expt. as C—F, fig. 16. C, *Vasto crureus* reflex, contralat. peron. popl. nerve, coil at 14.5 cm., freq. 39 p. sec. T, 0.4 sec. D, *Vasto crureus* reflex, contralat. peron. popl. nerve, coil at 16.5 cm., freq. 39 p. sec. T, 0.2 sec.

slightly stretched muscle, the reflex threshold for which stood at 22 cms. on the inductorium scale, gave on stretching the muscle 3 to 4 mm. more obvious reflex contraction at 23 cm. on the scale. The favouring influence of slight stretch in this muscle is evident also in the ipsilateral spinal reflex

of the muscle; also in its reflex rebound contractions of central original ensuing after reflex inhibition.

Examples of favourable influence of stretch of the extensor muscle on the reflex responses of the muscle.

Weight of cat.	Muscle.	Passive stretch, muscle length in mm.	Passive tension.	Tetanus height in mm.	Tetanus tension.	Tetanus tension + passive tension.	Stimulus.	Magnification by myograph.
grms.			kilos.		kilos.	kilos.	cms.	
1750	Vasto crureus	Slightly taut	0.1		0.65	0.75	14.5	40
		75						
1750	"	75+9	0.45		2	2.45	14.5	40
1750	"	75	0.1		0.4	0.5	15.2	40
1750	"	75+11	0.75		1.75	2.5	15.2	40
	Rectus femoris	92+3	0.16	34	1.8	1.96	12	68
	"	92	< 0.05	19	1.02	1.07	12	68
	"	92	< 0.05	12	0.68	0.73	13	68
	"	92+4	0.2	27	1.7	1.9	13	68
	"	92	< 0.05	9	0.5	0.55	13.8	68
	"	92+6	0.27	23	1.22	1.49	13.8	68
	"	92	< 0.05	8	0.45	0.50	13.8	68
	"	92+4	0.17	23	1.22	1.39	13.8	68
	"	92	< 0.05	9	0.5	0.55	13.8	68
	"	92+8	0.3	27	1.5	1.8	13.8	68
	"	92	< 0.05	7.5	0.4	0.45	14	68
	"	92+6	0.24	20	1.13	1.39	14	68

Powerful as this influence is, it does not, however, change the essential form and peculiarities of extensor-reflex ascent, or assimilate them to those of the mn. tetanus or the spinal flexor-reflexes.

Another characteristic of the decerebrate extensor's crossed reflex contraction is the height (tension) ratio between the single-shock reflex and the tetanic reflex. In the mn. reactions of this extensor muscle the ratio twitch-height (tension) to tetanus height (plateau-tension) (figs. 14, 18) is not in our experience markedly dissimilar from that found, as above mentioned, for the two flexor muscles. For the main divisions, viz., crureus, vasti and rectus, of quadriceps extensor, this mn. twitch/tetanus ratio has it is true in our experience varied considerably, as in our hands for the flexor muscles. One factor in this variation we incline to attribute to differences of initial tension (fig. 18). It would seem from observations mustered in our records that low initial stretch may somewhat heighten, and high initial tension somewhat lower the ratio. Further, the ratio may not be the same for all four divisions of so large and complex a muscle



as the quadriceps. The ratio in our observations has ranged between twitch-height/tetanus-height 40/100 to twitch-height/tetanus-height 16/100. But in the decerebrate crossed reflex (figs. 15, 17, 19) of this muscle the range of the ratio single-shock reflex-height/tetanic reflex-height runs between 0/100 and

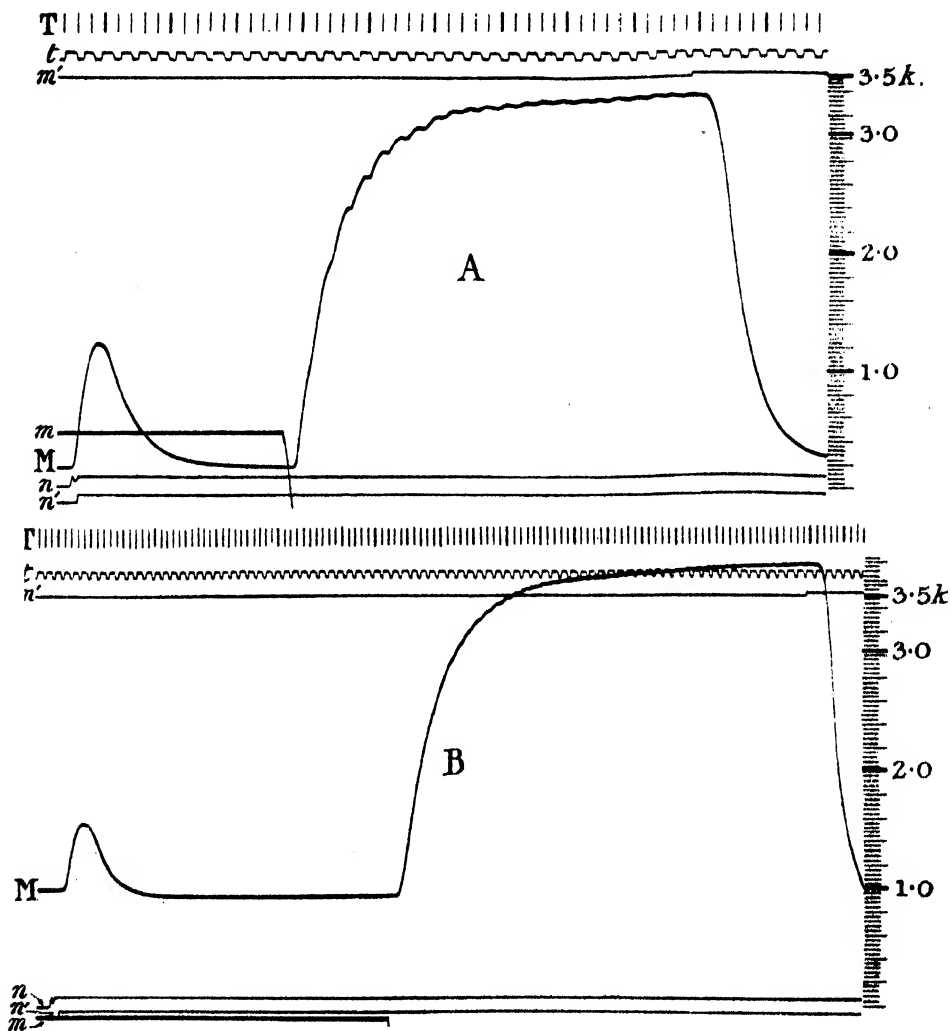


FIG. 18.—A, *Crureus* and *Vastus int.*: motor nerve, coil at 17.8 cm. (just maximal), freq. 38 p. sec., s.s. followed by tet. Initial stretch of muscle low. Myograph  $\times 38$ . B, *Crureus* alone; motor nerve, coil at 22 cm. (? maximal), freq. 38 p. sec., s.s. followed by tet.: initial stretch considerable giving init. passive tens. of 1000 grms. Recording surface travelling only half as fast as A. Myograph  $\times 38$ . Cat smaller than in A. Calibration of tension at side.

11/100 (fig. 20). It is, in fact, of a different order from that of the mn. reactions; and still more so from that of the spinal flexor-reflexes where

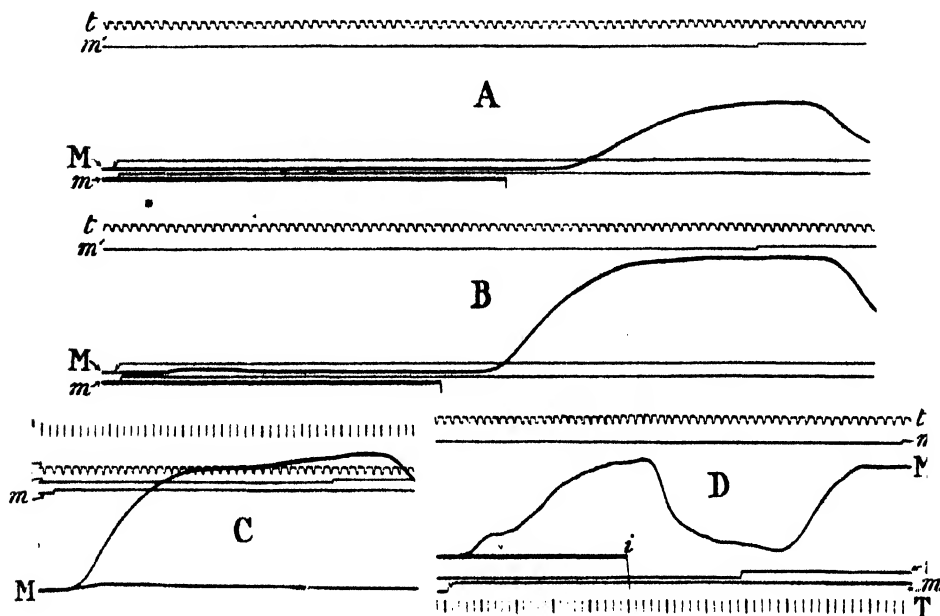


FIG. 19. A and B.—*Vasto crureus* reflex, contralat. peron. popl. nerve. A, coil 15 cm., freq. 38 p. sec., s.s. followed by tet.; B, ditto but coil at 14 cm.; to show s.s./tet. ratios. C. *Crureus* reflex, contralat. peron. popl. nerve, coil 14.8 cm., freq. 49 p. sec. s.s. reflex and tet. compared with same starting point. s.s. stim. obtained by unshort-circuiting serial stimuli for 0.02 sec. at same coil strength, i.e. 14.8 cm. T, 0.02 sec. D, *Vasto crureus* reflex from contralat. peron. popl. nerve, coil 16 cm., freq. 49 p. sec., inter-current inhibition by stim. of ipsilat. peron. popl. nerve, coil 16 cm., freq. 90 p. sec. T, 0.02 sec. i, inhibit. stim. begins; inhibit. stim.'s end shown by signal line i'.

the ratio single-shock reflex/tetanic-reflex runs between 33/100 and 70/100 in semitendinosus and between 40/100 and 75/100 in tibialis anticus.

Employing for interpretation of the isometric myograms of this extensor-reflex the just mentioned data, and applying the same principles as used above for the spinal flexor reflexes, certain further differences between those two flexor reflexes and this extensor-reflex have to be borne in mind. (1) The motor discharge of the extensor-reflex unlike that of spinal flexor-reflexes does not correspond in rhythm with the faster rates of external stimulus. Nor does it at those rates exhibit in our records any marked rhythm at all, although our registration is competent for rates of rhythm up to the limit set by complete confluence of contraction waves in the extensor muscle's fibres.

That limit in our records lies somewhere close above 90 per second. Therefore the contraction-wave rhythm in the reflex must either be consistently asynchronous in the different fibres of the muscle's mass, or be higher than 90 per second; perhaps it is both. The tetanic summation-time," i.e., the

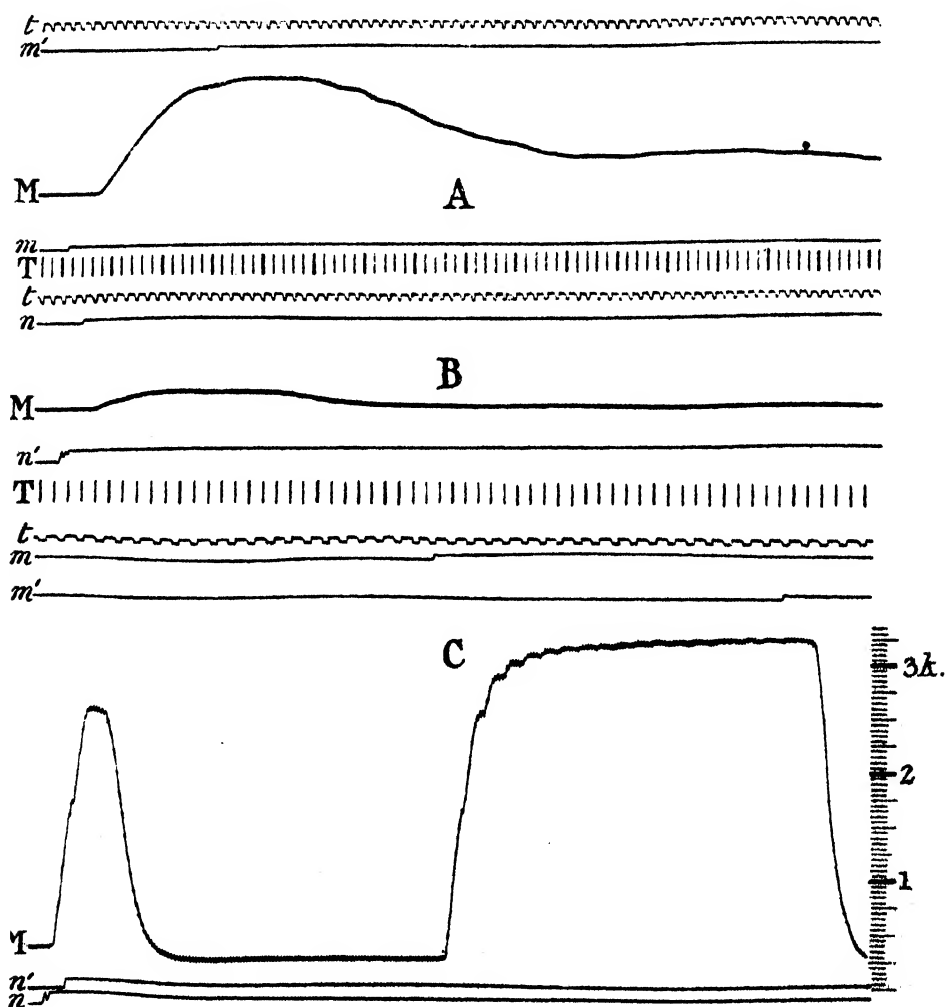


FIG. 20.—A and B.—*Vasto crureus* reflex; contral. peron. popl. nerve; consecutive obs., coil at 16 cm., freq. 49 p. sec. In A, thirteen shocks delivered, in B two (three) shocks. B's contraction tension about 13 per cent. of A's. C. *Vastus externus*: motor nerve, coil 18.5 cm., freq. 38 p. sec., delivery of two shocks followed by series of 19. Two-shock reaction develops a tension about 80 per cent. of tet. plateau tens. Cat wt. 2100 grms. T, 0.02 sec.; small vibs. in myograph record =  $1/250$  sec. Calibration scale of tension at side, in kgms. Myograph magnifies tendon movement 162 times.

ascent-period for the muscle's mn. tetanus under 90 per second stimulation is about 0.12 second (fig. 3, A). If, therefore, as seems likely, the rhythm of the motoneurone discharge in this reflex is more frequent than 90 per second, the muscle-fibres engaged should in their contraction under the reflex develop their tetanic plateau-tension in something like 0.1 second after commencement of their contraction. But observation shows that the duration of the ascent-period of the reflex contraction is commonly three and four times as long as that, and is sometimes thirty to forty times as long. (2) The after-discharge in this reflex is, though variable, usually much greater and more enduring than in the spinal flexor-reflexes (fig. 17, C). In those latter, the after-discharge, while also variable, is, in our experience, always "fractional," *i.e.*, never, as "terminally" (T. Graham Brown (12)) exhibited, involves synchronously the totality of the motoneurone group activated by the reflex. But in this extensor-reflex the "terminal" after-discharge still, after deduction of a period equal to the latent period of the reflex, often continues the full plateau tension of the contraction for a considerable time. Such after-discharge appears, therefore, to include at first, the entire quota of the group of motoneurons activated by the reflex. Instead of being, as in the spinal flexor reflexes, a "fractional" after-discharge in this extensor-reflex there is, for a time, an after-discharge from the totality of the motoneurone group that has been activated by the reflex.

A further discrepancy between the ascent-periods of this crossed reflex and of the mn. tetani of its muscle is the fundamental unlikeness of their curves of ascent. Besides the former's excessive duration and, as compared with the s.s. reflex, excessive height, its ascent is commonly sigmoid, occasionally concave (fig. 12, C), upward even throughout the greater part of its course, or sometimes becomes nearly rectilinear. The ascent of the mn. tetanus can, of course, be given a sigmoid or concave upward or fairly rectilinear form by progressively varying the intensity of the individual shocks composing the serial stimulation during the course of the stimulation. Thus even the single intercurrent augmentation of the serial stimulus used for producing incremental contraction in the mn. tetani and spinal knee flexor-reflex impresses a roughly sigmoid form upon the tetanic ascent (fig. 2, C). But the comparison drawn here is based on equable stimuli of constant intensity and frequency for both reflex and mn. tetani.

An inference which the characters of the contraction-response in this reflex allow is that while the number of muscle-fibres which the reflex response employs at commencement is practically always quite small, the reflex as it

proceeds, brings into action fresh additional muscle-fibres, and does so successively and more or less continuously and gradually under mere continuance of the unaltered external stimulus. The evidence shows that this process of accretion or drawing into action of contractile units over and above those already involved, after a variable and sometimes stepped progress declines gradually, either under continuance of the external stimulus or after withdrawal of the external stimulus. The course of the accrescent process may, however, under continuance of the external stimulus sometimes endure through even three, four or five seconds. With the mn. tetani the ascent-time tends to be longer with stronger stimuli than with weaker: with the reflex tetani the ascent time is often longer with weaker stimuli than with stronger.

The process just described for the reflex as expressed in muscle-fibre contraction, can, we infer, be accepted for the motoneurones of the extensor muscle. The external stimulus in this reflex, therefore, begins by exciting a relatively small, even very small, number of those motoneurones; but under mere continuance of the unchanged stimulus, the number of motoneurones reflexly activated increases progressively through relatively long periods, until it amounts to many times the number initially engaged. And this recruitment sometimes (Fig. 12, F) acts in steps, and sometimes (Fig. 17, D) goes out of action similarly.

The glimpse thus obtained of the happenings in the reflex centre has to take account of the conditions of the motoneurone. Thus, where, as often in this reflex, the after-discharge is "total," cessation of the external stimulus leaves all those motoneurones which have been already activated by the reflex still actively engaged for a time in the discharge of impulses presumably at high frequency-rate. This being so, a lapse of the continuance of the external stimulus for a short period, say for a tenth of a second, will cause a check in the ascent (*i.e.*, in the rise of contractile tension) of the reflex contraction, but the tension will not in that short time sink below its level already reached. Fig. 9 in our previous paper (1) exemplifies this. It shows that on lapse of the external stimulus the rising tension continues its rise for a period equal to the latent period of the reflex and then runs horizontal at the attained level. That is to say, after a short lapse of the external stimulus renewal of that stimulus will find the motoneurones which the stimulus had already activated still engaged in a discharge originally started by itself, the external stimulus, but now self-operated by the central mechanism. All the motoneurones activated by the reflex at the moment when the external stimulus lapsed

being thus on renewal of that stimulus still occupied by discharge and thus precluded for the time being from further response to the stimulus, if any further increase of the reflex contraction occur at renewal of that stimulus such increase must attach to activation by the stimulus of fresh additional motoneurones. As the fig. shows, the renewal of the stimulus does result in increase of the reflex contraction; this latter renews its ascent from the level at which it was interrupted, and continues it until final withdrawal of the stimulus. Since the observation is applicable throughout the ascent, the inference is that throughout the ascent the reflex is, under the support of the unchanged external stimulation, continually drawing, so to say, into its vortex of activation additional numbers of fresh motoneurones.

Adopting the conclusion that in this extensor reflex the isometric ascent curve in its various sub-forms, sigmoid and other (*v. supra*), signifies addition of fresh contracting units and inferentially of fresh activated motoneurones the several forms assumed by the course of the ascent indicate the various time relations exhibited by the progressive involvement of additional motoneurones during the development of the reflex. That process may, for convenience of statement, be designated "recruitment." The various sub-forms of the ascent show that this recruitment commonly increases up to a maximum regularly and gradually and then progressively diminishes. It is, nevertheless, sometimes irregular and sometimes shows little change in amount even throughout long periods, even up to the delivering of 150-200 consecutive stimuli. In all its forms increase of frequency and especially increase of intensity of the stimulation appear to accelerate and intensify it.

Recruitment, present in spinal extensor reflexes, has not been detectably so in spinal flexor reflexes. If present in them, it has been so in degree too slight to allow unequivocal statement of it. This difference between those reflexes and that of the crossed extensor is tantamount in effect to mere prolongation of the stimulation being a factor in the intensity of the latter but not of the former, providing only that as regards the former the brevity of the stimulation be not so great as to fall short of the net tetanus summation-time of the peripheral muscle-fibre.

That recruitment thus characterises the one type of reflex and not the other, can hardly be attributable to difference in the afferent fibres employed for excitation of the two, since the afferent nerves used for both have been the same. That it does not arise from the motoneurones themselves seems indicated by the circumstance that recruitment does not in our experience attach to the knee-extensor's ipsilateral spinal reflex. A main feature of contrast between

the conditions for the two types of reflex, crossed knee extension on the one hand and ipsilateral knee flexion and ankle flexion on the other, lies in the former's being favoured by the decerebrate preparation. On the knee extensor's motoneurones the hind-brain is known to exert marked influence, as evidenced by the heavy depression, even abrogation, of the crossed extensor reflex which follows severance from the hind-brain by spinal transection. This consideration suggests that recruitment is favoured by interplay between the spinal centres and the hind-brain. The recruitment may conceivably be regarded as the expression of a central "addition latente" (Richet (13)) of extreme degree. But if so, the implication that mere repetition of impingement at the final synapse accounts for the recruitment must be made with caution, because the time relations of the reflex ascent seem in many cases beyond what such "addition latente" exhibits elsewhere, and would require for some of the motoneurones latent summations of impulses due to several hundreds of successive stimuli.

The tension-reascent of the extensor reflex contraction ensuing after intercurrent inhibition—the original excitatory stimulus still continuing unaltered—frequently shows the sigmoid form (fig. 19, D). The recovery of the reflex contraction in these cases also seems to involve "recruitment."

The crossed extensor decerebrate reflex exhibits markedly the reflex characters sometimes figuratively termed "inertia" and "momentum" (fig. 21, A, B). Recognition of the process of recruitment permits a clearer view of these characters. Initiated by the stimulus to the afferent nerve, the recruitment seems to start focally on a basis so restricted that its actual commencement often probably escapes immediate detection in the myogram. But once started, it progresses, even after withdrawal of the external stimulus, for a period not less than, often somewhat longer than, the apparent latency of commencement of the reflex. It thus has characters figuratively analogous to inertia and momentum. Recruitment is, however, always absent from the true after-discharge often so prolonged in this reflex (*e.g.*, fig. 17, C).

Reflex inhibition by the ipsilateral afferent nerve cuts short the recruitment and the after-discharge (fig. 19, D). This inhibition develops its action after a latent period far shorter than the latent period or the "momentum" period of the recruitment process itself. A main seat of the recruitment process must, therefore, lie up-stream in the reflex-arc above the central locus of incidence of the inhibition itself, if this latter process act merely as a block.

And however this latter process act, the main seat of the recruitment process cannot lie further down-stream than the locus of the inhibition.

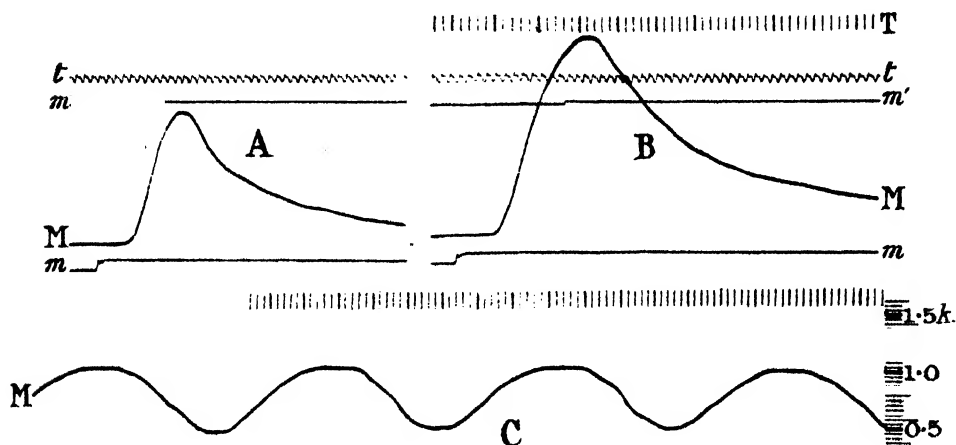


FIG. 21.—A and B. *Vasto crureus* reflex, contral. peron. popl. nerve, coil at 14.2 cm., freq. 47 p. sec. In A six stimuli delivered, in B eleven. T, 0.02 sec. C. 'Spontaneous' stepping in decerebrate deafferented *vasto crureus*; isometric contractions (ascents) and relaxations (descents). Myograph magnifies 38 times. T, 0.02 sec. Large cat. Calibration of tension in kgm. at side.

The abruptness of the arrest of contraction produced by the reflex inhibition, approximating in speed and often in completeness to the cessation of an mn. tetanus itself, indicates that the ipsilateral reflex inhibition of the extensor falls with striking approach to simultaneity on all the motoneurones which it affects. In this, this inhibitory reflex resembles its counterpart\* the ipsilateral reflex excitatory to the flexors. In the latter, as shown by the likeness of the single-shock reflex to the mn. twitch and of the development of the reflex tetanus curve to that of the mn. tetanus the reflex excitation takes effect with approximate simultaneity on all the motoneurones which the reflex engages. Although, therefore, the influence on the extensor is inhibitory but on the flexor excitatory, the ipsilateral reflex in both of these its two aspects has the same above-mentioned character contrasting with that of the crossed extensor-reflex.

The isometric records of certain other contractions due to spinal discharge, besides those of the crossed extensor-reflex, exhibit forms of ascent plainly suggesting an accrescent process. Thus, the rebound contraction which

\* That the latent period of the inhibitory reflex is regularly somewhat greater than is that of the excitatory, see J. M. D. Olmsted and W. P. Warner (15), does not affect the consideration here followed.



tends so commonly to ensue in the extensor after cessation of an inhibitory period sometimes shows such a form of ascent. Again, isometric records of the rhythmic contraction of the fully isolated and deafferented vasto crureus in the "spontaneous" stepping which sometimes makes its appearance in the decerebrate preparation in absence of all obvious external provocation exhibits a sigmoid ascent (fig. 21, C) for each step. The approximation of this "spontaneous" (T. Graham Brown (14)) stepping to a natural act suggests the sigmoid form as a natural feature of this phase of the rhythmic contraction for locomotion. But between these instances and the extensor-reflex examined is the difference that whereas the former are reactions to stimuli whose frequency and intensity-course are unascertained, the latter reaction is to a stimulation whose frequency and intensity are known and remain constant throughout its employment for excitation of the reaction.

To summarise regarding the crossed reflex of the knee extensor :

(1) The ascent duration of this type-reflex is longer than that of the fore-going flexor-reflexes, and is sometimes even ten, twenty times or more longer than that of the mn. tetanus of the extensor muscle itself. We infer that this is due to the "recruitment" by the reflex of fresh motoneurons in its course; and that the various forms, sigmoid and other, assumed by the ascent and plateau are due to the same cause.

(2) As with flexor mn. tetani: (a) lesser frequency of stimulus rate, or (b) greater intensity of serial stimuli, or (c) greater passive stretch prolongs the ascent and opens out the ascent-plateau corner. Of these (c) has greater influence on this muscle than on the flexor muscles examined. Greater passive stretch need amount to no more than 4 per cent. elongation of the total length of the muscle to double the tension developed during tetanus.

(3) These three factors (a), (b) and (c) have, in general, a similar effect on the crossed extensor reflex as on the flexor-reflexes examined, but the special character of the reflex curve as contrasted with theirs is still retained. A small increase in the initial passive stretch may render very apparent a single-shock reflex reaction of the extensor although the stimulus-strength remaining the same no reflex reaction to the single-shock is visible under lower initial stretch.

(4) With mn. reaction of the extensor, the twitch/tetanus ratio has ranged between 45/100 and 16/100, but with the analogous reflex reactions the ratio has ranged between 11/100 and 0/100; we infer that this discrepancy is traceable to "recruitment" in the reflex.

(5) As with the general form exhibited by the reflex curve so also with the

"inertia" and "momentum" of this reflex, these are features attaching mainly to the process of "recruitment."

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*The Basal Metabolism of a Growing Pig.*

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*Introductory.*

The experiments to be described in this paper are a continuation of the comprehensive investigation of the course of metabolism in swine foreshadowed by Dr. J. W. Capstick and Prof. T. B. Wood in their paper read before the Royal Society last year (1).

The instrument employed has been the same, erected by Dr. Capstick (2), which was used by the authors mentioned. It has been improved since the appearance of the memoir cited, by the addition of resistance thermometers in the exit ventilating tube, which are used for obtaining a continuous record of the temperature of the outlet air on a thread recorder similar in every way to the one employed for plotting the difference in temperature between the inlet and exit water of the circulatory system; and of an arrangement, operated photographically, whereby the height of the water surface in the manometer tubes is traced continuously on to a strip of sensitised paper fixed to the surface of a revolving drum. These recording arrangements are not usually used *in lieu* of personal observations, which are still continued by night as well as by day while an animal is in the calorimeter. The only occasions when a continuous automatic record has been used for computation has been when the restlessness of the animal rendered personal readings impossible. This method was not used for any of the experiments described in this paper, the only use made herein of the recorders has been for the recovery of a few lost readings, as if the pig is disturbed by the observer entering the room the outlet thermometers immediately rise before readings can be taken.

It has been found necessary to strengthen considerably the arrangements made inside the calorimeter to prevent the pig lying against the metallic side of the instrument. It was found that the previous arrangement of an internal cage of crate slats and wire netting was useless as a permanency; the animal soon made considerable rents in the wire netting and broke some of the slats. This, of course, does not allow of his lying against the side, but it has been our consistent experience that if there is any moveable object in the calorimeter the pig spends much of his time by day and night in trying to

wrench it free. For some time the cage was omitted on this account, but it seemed possible that the greater rate of cooling where the hog's body was in contact with the side might cause a rise in the metabolism akin to that caused by a fall in the external temperature. The instrument has therefore been fitted with a set of strong iron railings fixed in sections to the moveable flooring.

The pig used was a pedigree Large White, from the University Farm, Gravel Hill, Cambridge; bred by Mr. K. J. J. Mackenzie. It was a male, born March 11, 1922, and castrated April 25, 1922. It was weaned April 28, 1922, being at that time 25 lbs. in weight, and was brought down to the laboratories on May 9 in that year, *i.e.*, at the age of 59 days, and experiments were commenced as soon as it had had an opportunity to settle down in its new environment. They have been continued to the present time.

The hog has been kept in an out-door pen with wattle shelter until the beginning of this year; when concrete was laid down, as the condition into which this and two other pigs used for experimental purposes had got the pen was becoming bad for their health. At the same time, a more solid wooden shelter was erected. For a few days before an experiment is started the pig is normally brought indoors and inhabits a small pen in the basement of the School of Agriculture; it has also been the custom to bring him in during very cold weather.

As to food, Dr. Capstick informs me that he found the rations supplied to the pig he used, 7 lbs. per day on the average, caused it to grow rather rapidly, and I therefore used somewhat less than this. Throughout the experiments the animal's diet has been meal mixed with about three times its weight of cold water. The meal has been a mixture of 2 parts barley meal, 2 parts sharps and 1 part bean meal, and the quantities of this supplied to the animal during the times when it was not in the calorimeter are shown in Table I.

On the days on which an experiment was to commence the animal was placed in the calorimeter soon after nine in the morning and fed there with half its ration for the day; the other half was fed to it at about four o'clock the same afternoon. This treatment appears to conduce to quietness during the first night of the experiment, and has made it possible, in two cases, to obtain readings from which the metabolism during the first few hours after food can be computed.

The usual routine during the first months of life of the hog was that he should spend one week fasting in the calorimeter, and that this should be followed

by two weeks in the open on full diet. Towards the middle of the series of experiments the curves of descent to basal became somewhat uncertain, and the time between experiments was lengthened in case the hog might be finding the former regime too strenuous. The curves improved, but it is not possible to say definitely whether this had anything to do with the improvement or not.

When in the calorimeter the pig received only water from 4 p.m. on the day he was put in until he was taken out at the close of the experiment. He was supplied with fresh air by the ventilating mechanism at the rate of 300 to 400 litres per minute, which was enough to keep the inside reasonably free from gases such as carbon dioxide and ammonia, which might cause a rise of the metabolism.

Table I.—Rations of Hog when not in Calorimeter.

Dates.	Approximate age, days.	Approximate weight, lbs.	Lbs. of meal per day.
May 9, 1922, to May 11, 1922 .....	60	29	1
May 12, 1922, to May 22, 1922 .....	65	33	1½
May 25, 1922, to June 8, 1922 .....	82	38	2
June 9, 1922, to June 12, 1922 .....	91	45	3
June 16, 1922, to July 18, 1922 .....	113	55	3
July 22, 1922, to August 14, 1922 .....	146	80	3
August 18, 1922, to September 25, 1922 .....	179	110	3
September 30, 1922, to October 2, 1922 .....	205	125	3
October 3, 1922, to October 16, 1922 .....	212	140	3½
October 21, 1922, to October 31, 1922 .....	230	150	3½
November 1, 1922, to November 6, 1922 .....	242	160	4
November 11, 1922, to November 27, 1922 .....	253	170	4
December 3, 1922, to December 6, 1922 .....	268	185	4
December 7, 1922, to January 15, 1923 .....	290	200	4½
January 20, 1923, to February 28, 1923 .....	334	220	4½
March 4, 1923, to April 9, 1923 .....	376	245	4½
April 14, 1923, to April 24, 1923 .....	405	265	4½
April 24, 1923, to May 21, 1923 .....	448	285	5½
May 26, 1923, to July 2, 1923 .....	473	310	6

### *Experimental.*

The observations of the basal metabolism were made exactly as heretofore, that is to say, the metabolism per minute was calculated for a time, usually in the early hours of the morning, when the pig had been asleep for some hours. This procedure is necessary owing to the large water equivalent of the instrument, which renders it impossible to get satisfactory readings in the immediate neighbourhood of a time when the pig has been expending muscular energy. From these observations a curve was plotted for the duration

of the experiment, usually five days, and as a rule only one observation per day was selected, sometimes more, and occasionally none appeared to be sufficiently good for computation.

It was found, as Capstick and Wood (*l.c.*) had found, that the metabolism continued to descend until about 80–100 hours after the intake of food in the case of a grown animal. When the pig was younger he appeared to get down to his basal sooner. As the pig is at the present moment only slightly older than the one Capstick and Wood used was when they started, it has not been found possible to construct a composite curve of the same nature as that described by them, and I therefore give what one may perhaps call the *Curves of Descent* to basal for twelve of the thirteen experiments made. The eleventh, which was conducted November 27 to December 2, 1922, gave a set of points through which no reasonable curve could be drawn, all the observations were, moreover, about 20 per cent. *below* those of November 6–11. No explanation of this has so far appeared; the instrument has been quite satisfactory before and since, and the pig appeared in good health when he went in and when he came out. These results are omitted. Numerical particulars of the remaining twelve are given in Table II, and the curves are plotted in fig. 1. The curve of May 26, 1923, is abnormal and may indicate that the pig was in a low state at the beginning or sleepless towards the end.

All the observations were made at a uniform temperature of about 16.3° C. This was chosen because it is easy to maintain at all seasons.

Table II.—Observed Metabolism on Various Dates.

Date ..... May 25, 1922		Date ..... June 16, 1922	
Age ..... 75 days		Age ..... 97 days	
Weight ..... 28 lbs.		Weight ..... 37 lbs.	
Hours fasting.	Metabolism, cals. per min.	Hours fasting.	Metabolism, cals. per min.
15½	0.533	14½	0.907
43	0.492	43½	0.780
66½	0.468	65	0.698
—	—	88	0.694
Basal metabolism ..... 0.47		Basal metabolism ..... 0.70	

Table II—(continued).

Date .....	July 22, 1922	Date .....	August 18, 1922
Age .....	133 days	Age .....	160 days
Weight .....	65 lbs.	Weight .....	89 lbs.
Hours fasting.	Metabolism, calcs. per min.	Hours fasting.	Metabolism, calcs. per min.
13	1.294	14	1.523
38	1.099	19	1.461
62	1.028	64	1.239
83	0.930	91	1.221
Basal metabolism ..... 1.62		Basal metabolism ..... 1.62	
Date .....	September 30, 1922	Date .....	October 21, 1922
Age .....	203 days	Age .....	224 days
Weight .....	121 lbs.	Weight .....	141 lbs.
Hours fasting.	Metabolism, calcs. per min.	Hours fasting.	Metabolism, calcs. per min.
2	1.644	10	1.770
5	1.705	37	1.455
8	1.582	60	1.397
10	1.501	82	1.313
31½	1.320	104½	1.374
59	1.357		
78½	1.318		
103	1.359		
Basal metabolism ..... 1.33		Basal metabolism ..... 1.345	
Date .....	November 11, 1922	Date .....	January 20, 1923
Age .....	245 days	Age .....	315 days
Weight .....	153 lbs.	Weight .....	193 lbs.
Hours fasting.	Metabolism, calcs. per min.	Hours fasting.	Metabolism, calcs. per min.
12½	2.067	7	2.440
34	1.494	35	1.808
58	1.435	61	1.458
91	1.415	84½	1.448
104½	1.304		
Basal metabolism ..... 1.40		Basal metabolism ..... 1.40	

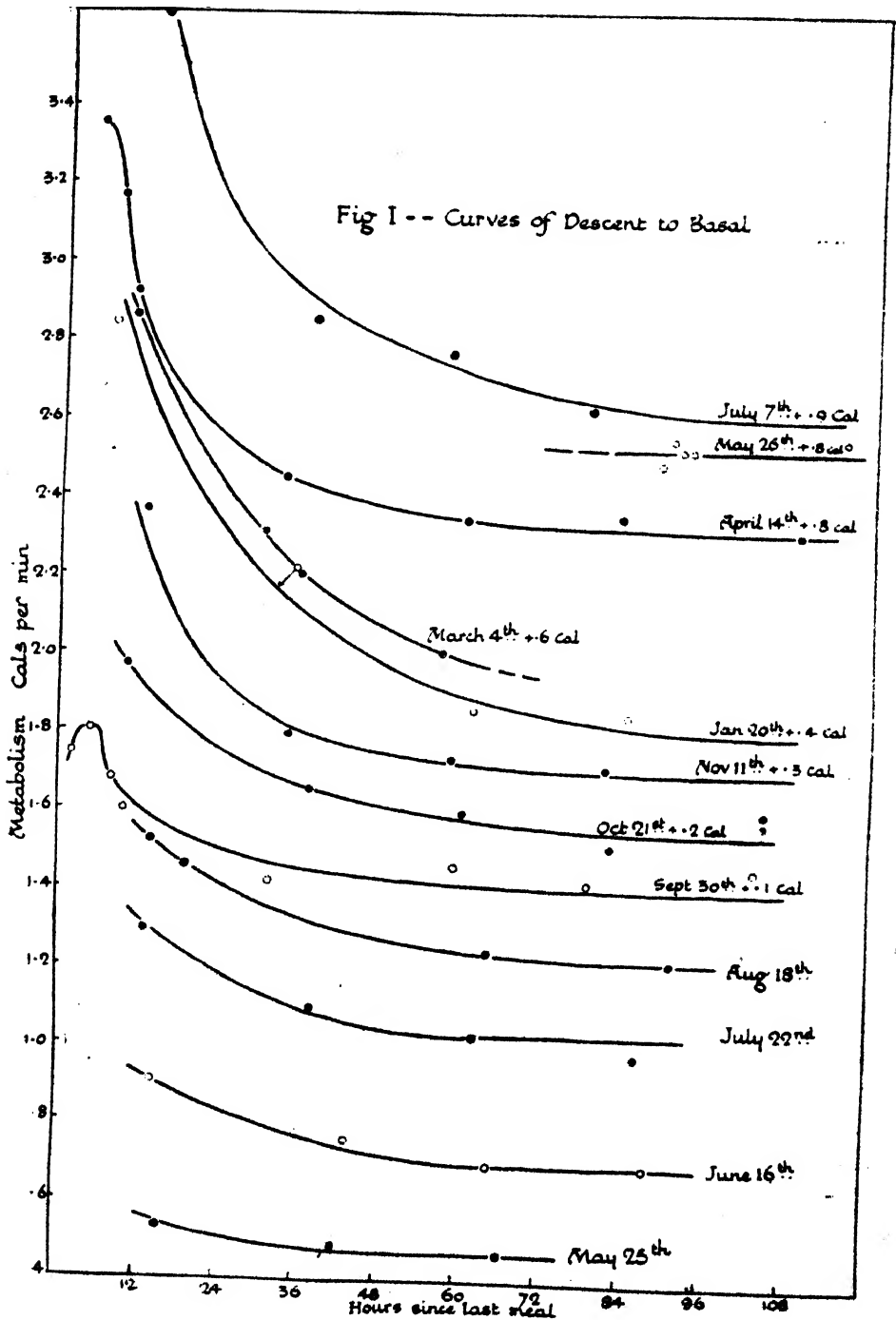
Table II—(continued).

Date .....	March 4, 1923	Date .....	April 14, 1923
Age .....	358 days	Age .....	399 days
Weight .....	224 lbs.	Weight .....	248 lbs.
Hours fasting.	Metabolism, cals. per min.	Hours fasting.	Metabolism, cals. per min.
10	2.260	5	2.557
30	1.713	8	2.365
35½	1.601	10	2.123
55½	1.454	33	1.047
—	—	60	1.554
—	—	83	1.558
—	—	109	1.521
Basal metabolism ..... 1.38		Basal metabolism ..... 1.52	
Date .....	May 26, 1923	Date .....	July 7, 1923
Age .....	441 days	Age .....	483 days
Weight .....	278 lbs.	Weight .....	303 lbs.
Hours fasting.	Metabolism, cals. per min.	Hours fasting.	Metabolism, cals. per min.
14	1.848	9	2.830
41	1.541	37	1.946
63	1.600	57½	1.870
88	1.691	78½	1.732
90½	1.758		
92	1.727		
93	1.730		
116	1.767		
Basal metabolism ..... 1.73		Basal metabolism ..... 1.71	

The path by which the metabolism approaches its basal value appears to vary in different cases, the curves of September 30, October 21, November 11 and April 14 being decidedly flatter than the rest in the region from 50 hours upwards. This shape appears more or less characteristic for this pig, but the curves of January 20 and March 4 are approximations to the composite curve of Capstick and Wood, so it is conceivable that the more rapid fall is due to short rationing ; it is proposed to test this.

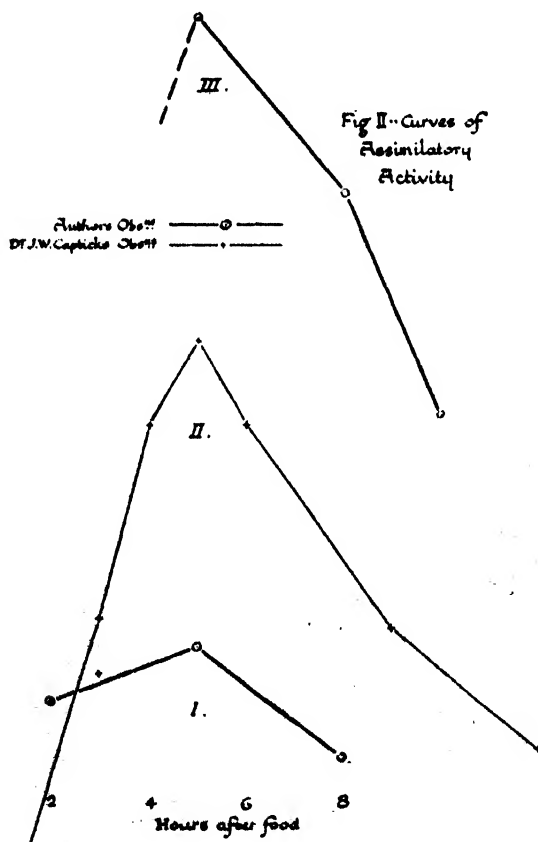
The irregularity of the lie of the points about the curves in the last quarter of 1922 is noticeable. As has been mentioned, the time between the trials





was lengthened about this time in the hope of improving matters, and the curves in fact improved ; but it may well be that the irregularity was due to some other cause. The pig was more restless at this period for some reason, rising five and six times nightly for micturition. This would account for high values but hardly for low ones.

Attempts are always made to get some measure of the course of metabolism during the early hours. For this reason the 4 p.m. meal in the calorimeter on the day of entry has been introduced, as it tends to make the animal more comfortable and so conduces to early sleep. On two occasions observations of this nature have been obtained, namely in the experiments of September 30 and April 14. Also by the courtesy of Dr. Capstick I am able to mention certain results of a similar kind obtained by him in somewhat greater number, in which a pig was kept in the calorimeter and fed at 6 a.m. and 6 p.m. on



a ration slightly below that required for maintenance. These observations are not consecutive on one day, the time has been reckoned up to 12 hours, which has then been regarded as 0 hours and a fresh start made. One of these observations in which the metabolism at 10 hours was only 0.07 cal. per min. above the basal has been rejected. These curves are shown in fig. 2, and data relating to them in Table III. I am myself responsible for the drawing of the curve connecting Dr. Capstick's observations.

Table III.—Particulars relating to Curves of Assimilatory Activity.

Curve.	Pig.	Date.	Age, days.	Weight, lbs.	Basal metabolism corrected to date.
I.	" Peter "	September 25, 1922 ....	198	121	1.35
II.	" John "	March 12-16, 1923 ....	340	217	1.38
III.	" Peter "	April 9, 1923 .....	390	248	1.52

One thing appears to be quite definitely established by the curves of fig. 2, namely, that the maximum of assimilatory activity is reached 5 hours after the ingestion of food ; in the case of Curve III, although there were no earlier observations, the curve was steady and rising up to the 5-hours' point. This is indicated by the dotted line. This result is in striking accord with the results obtained by Lusk (3) on dogs. As regards the general shape of the curve before and after this maximum, there appears to be no justifiable means of combining all three sets of observations on to a single diagram and running a smoothed curve through them. Failing this, I have run through Dr. Capstick's points what seems to be the most likely course. It is clear that the curve cannot rise on ingestion of food, even if this were pure glucose ; time must be given for this to reach the resorptive system and generally for the conversion of the food into an assimilable form. There will therefore be a fall at first, followed by a rise as carbohydrates, and, later, fats and amino-acids yield their quota of heat in the process of katabolism. The justification for selecting the higher 9-hour and lower 12-hour observations is that there is no probability of any such " shoulder " in the curve as would otherwise be formed at 12 hours.

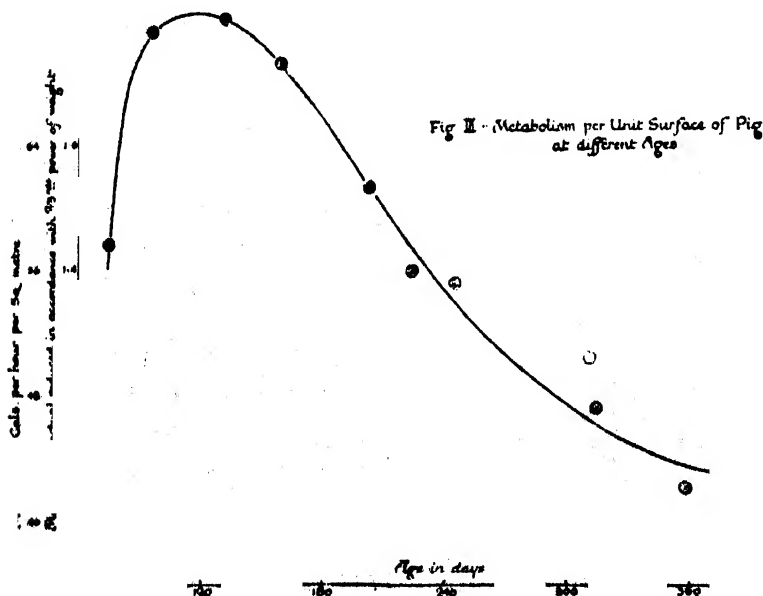
To come now to the main matter under consideration, the basal metabolisms obtained from the curves require to be reduced to calories per square metre in order that they may be comparable one with another. For this purpose there is only Meeh's equation (4) available, viz. :—

$$\text{Surface} = k (\text{weight})^{2/3}.$$

The value of the constant  $k$  in this equation when the animal is a pig is given by Voit as 9.02, but we may certainly conclude that the third significant figure is not in fact significant at all. Rubner gives 8.7 for this constant. For human beings the formula of Meeh has been much improved upon by the Du Bois (5), but for pigs no such improved formulæ exist. For practical purposes we may take  $k = 9$ , but it is well to remember that this may not be strictly constant at all ages or for all breeds of pig.

It is convenient to reduce all metabolisms to a weight of 150 lbs., since the surface of such a pig works out at 150 sq. decimetres, with an accuracy of *ca.* 1 per mille, well within that of the constant employed in the computation; hence to obtain metabolism in calories per hour per square metre, which is the standard which has been used largely for men, we have only to multiply the metabolism so reduced by 40. The curve in fig. 3 has been plotted in this way.

The curve is higher as a whole by about 12 per cent. than the corresponding curve deduced from a large number of observations of various workers on man by Du Bois (6); this may or may not be correlated with the higher normal body temperature of the pig. For the rest it resembles Du Bois' curve very much in shape, and in the fact that the metabolism falls eventually to a value somewhat above half that reached at the maximum attained in early youth, 4 months age in the pig, 5 years in the human subject. The slight rise on the



down grade found at puberty in man is not unnaturally absent from the present curve as a castrated animal was used.

The three unshaded points represent the three observations of Capstick and Wood (*l.c.*) reduced to the number of cals. per sq. metre, exactly to what extent the curve given by the animal used by them would have differed from the present one is uncertain. Armsby and Fries' (7) results, with a full blood and scrub steer, appear to show that a considerable difference is possible. While Tigersted (8) differs essentially from Du Bois in finding a slow diminution of the metabolism per unit area from youth to maturity.

### *Theoretical Discussion of Results.*

Even a cursory examination of the curve of fig. 3 stamps it as one of a class very familiar in biological sciences in connection with phenomena showing optimal points in regard to variations of temperature or other physical factors, the usual type is like the present one but laterally inverted. Now the explanation of such curves can usually be found in two effects tending in opposite directions, *e.g.*, rate of plant growth increases with temperature until at temperatures above 37° C. it stops increasing and is rapidly inhibited by the onset of the phenomena of heat rigor; so also the rate of enzyme action usually increases with temperature until slowed down by lack of sufficient supply of substrate or gradual destruction of the enzyme.

What explanation can be suggested for the curve with which we are now dealing? Armsby (9) sought one in the greater muscular activity of young animals; but while this may account for a portion of certain integrated effects, it is by no means the whole story, since all observations of the present series were taken when the pig was asleep and had been so for some hours.

If we consider the development of active tissue *subsequent to birth*, we find a considerable divergence of views among physiologists. Halliburton (10) in his text-book states that, apart from the longitudinal splitting of the muscle fibres, there is a definite amount of new growth due to development of the so-called "sarcoplasm" which are embryonic cells pre-existing in the interstices between the original fibres. Lungwitz (11) says nothing of the sarcoplasm, but only indicates that the mitotic process of nuclear fission connected with the splitting up of the fibres gradually ceases; while, according to Godlewski's view (12), the skeletal muscle fibres of sucklings are formed chiefly by fusion of several myoblasts and only a few by growth of individual cells.

Each of these theories seems *capable* of accounting for the rise of metabolism

in early youth, but the last seems most unlikely to produce such an effect, while the second requires the assumption of some set-back in the process of nuclear fission due to birth or other cause, a thing not in itself unlikely but introducing an additional assumption. If the first view is correct, it seems to contain within itself the germ of an eventual explanation of the phenomenon. The sarcoplast, quite apart from its power of living, and metabolising a certain quantity of nutritive material in the process, evidently possesses, over and above this, the power of growth and development which presumably require energy for their accomplishment. Now, although some energy may be stored in the new tissue formed, some will inevitably be wasted as heat. It seems, therefore, not improbable that it is just this heat which is given off in addition to the normal heat of metabolism when an animal is young. It seems fair to assume that the sarcoplasts will each begin their development as soon as conditions are suitable, and the suitability of conditions will certainly depend to some considerable extent on the space available; thus we may expect an ever-increasing rate of development during the early days of life; after a time, however, the supply of undeveloped sarcoplasts will become exhausted, and there will remain simply the as yet incompletely exhausted growth activity of those sarcoplasts which have started on the path of development. While more and more fresh sarcoplasts were beginning their development, the additional metabolism above the normal basal per square metre would rise rapidly as an accumulation curve. When the last had developed, the curve, remaining an accumulation of the extra metabolism of the cells, would fall gradually as these ceased to generate more than a normal basal heat.

The daily metabolism of the pig works out at 1,032 cals. per square metre per diem, a result which compares well with that obtained by Capstick and Wood, namely, 906 cals. per square metre per diem. The animal used by them had received considerably more food than my pig, and was presumably fatter; hence, if the constant of Meeh's equation is reduced with fattening in swine as in cattle, we should expect a higher value for the leaner pig, quite apart from any heat insulation due to fat in the sub-dermal layers. Higher values have been obtained by various workers in this field, notably Voit (13), 1,075 cals., mean from Meissl's figures (14); and Tangl (15) a range of values for different pigs varying from below 900 up to more than 1,200 cals. per square metre per day. It may be that there is a real variability in this respect, variations of 10 per cent. have been found to be normal in men by Gephart and Du Bois (16); these investigators considered variations exceeding 15 per cent. to be demonstrably abnormal in all cases.

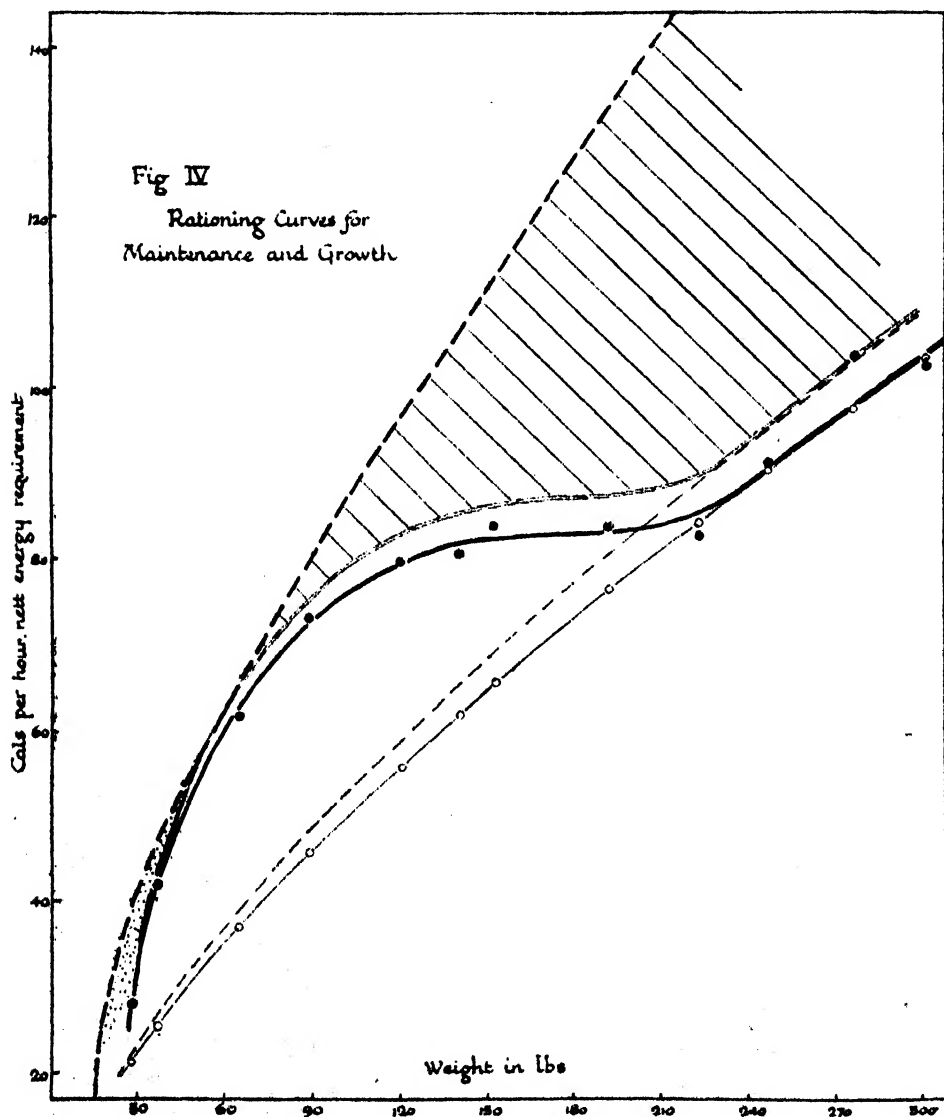
*Practical Results.*

From the practical point of view a matter of some import appears to arise. It has been customary to feed a certain number of units of nett energy daily for maintenance; where growth has also to be provided for a larger amount of energy is fed per head at any given weight of animal, but in either case the quantity is varied as the two-thirds power of the weight, i.e., as the surface of the animal. Now the basal metabolism under any conditions is precisely the nett energy requirement under those conditions for maintenance, and for growth if growth is present. It appears, therefore, that the feeding for maintenance and growth, without fattening, should not vary according to the two-thirds power of the weight, since the metabolism per unit area is not constant over the growth period.

In fig. 4, I have plotted with a thin continuous line the ration in cals. per hour nett energy required for the pig used in the experiment, calculated on the basis of a constant requirement of 43 cals. per hour per square metre surface. The thin dotted line shows Armsby's rations for maintenance of pigs on the same basis. The difference between the two is doubtless due to three factors—(1) Armsby's maintenance ration includes a certain amount of muscular movement; (2) Armsby's figure is the mean of a number of animals, while my experiment was carried out on one only; (3) The temperatures may not have been identical.

The heavy continuous line in fig. 4 shows the requirements for growth and maintenance as determined by experiment on the basal heat production, and hold, therefore, only for my animal at rest. We should expect, however, that the actual ration of practice would be got by adding to this an empirical correction for muscular movement, and variation of my pig from the mean, &c., which, for want of data, I take as the difference between Armsby's and my own maintenance curves. Making this correction, we obtain the double-line curve, but the practical rationing curve for feeding purposes where maintenance and growth is required is shown, after Armsby, by the thick dotted line. This ration is not claimed as one which will supply growth and maintenance and entirely prevent fattening; some accumulation of fat is obtained with it, and, in view of the experiments to hand, it seems likely that the extra amount of energy above that required for growth and maintenance which is included in Armsby's ration at weights exceeding about 75 lbs. (shaded in figure) would be stored in part as fat. The fact that the two curves touch one another at weights about 60 lbs. shows that *if we restrict ourselves to two-thirds power*

curves, the one deduced from Armsby's observations is the lowest which will provide for growth and maintenance at all weights.



Practical experience appears to bear out the above theory—I am informed that it is impossible to fatten pigs of the large white breed until they are above 75 lbs. in weight, and the explanation would appear to be that they cannot be persuaded to consume *more* food than is sufficient for growth and maintenance until this point is passed, possibly due to the smallness of the stomach of the



newly weaned pig. When the piglings are on their mother some fattening takes place, perhaps accounted for by the stippled area.

It seems reasonable in the circumstances to predict that a pig of this breed fed on the rations represented by the double-line curve would grow and maintain itself, but would not fatten, while one fed on Armsby's ration would begin to fatten slightly as soon as it began to exceed 75 lbs. weight. Further experiments by successive slaughter are to be carried out shortly in the School of Agriculture with a view to testing this deduction.

The experiments described above were made, until November, 1922, under the supervision of Prof. Wood, on his vivisection licence; subsequent to that date the author has held his own licence.

Before concluding, it is my privilege and pleasure to express my thanks to Prof. T. B. Wood, C.B.E., M.A., F.I.C., F.R.S., for the continued interest he has displayed and the expert advice which has always been at my disposal; to Dr. J. W. Capstick, O.B.E., M.A., whose guidance in the use of the instrument he constructed has been invaluable, and to Capt. J. S. Morgan, M.C., B.A., for assistance in the work of observing through the nights and the efficient way in which he has cared for the health of the pig during the intervals between experiments.

### *Summary.*

Various alterations in the calorimeter described in 'J. Agric. Sci.' vol. 11, pt. 4 (1921), are mentioned, and certain modifications of technique described.

The basal metabolism of a pig has been measured at various ages from 75 days upwards, and it has been shown that in the pig, as in human beings, the metabolism per unit area is greater in mid-youth than at any other time of life.

Experiments are adduced to show that the metabolism after the ingestion of food reaches a maximum after 5 hours and then declines.

The curve of basal metabolism showing its variation with age is discussed, and reasons are given for thinking that this increase of metabolism in youth is directly ascribable to growth.

The rationing of pigs for maintenance and growth is discussed, and it is concluded from the experimental results achieved that the curve of rationing for growth and maintenance, without fattening, cannot possibly be a two-thirds power curve.

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*The Kinetics of Hæmoglobin. II.—The Velocity with which Oxygen Dissociates from its Combination with Hæmoglobin.*

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(Communicated by Prof. J. N. Langley, F.R.S.)

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*Analytical Studies on the Factors Causing the Sexual Display in the Mountain-Newt (Triton alpestris).\**

By WALTER FINKLER, University of Vienna.

(Communicated by Prof. E. W. MacBride, F.R.S. Received June 7, 1923.)

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I.—*The Sexual Display of the Mountain-Newt.*

Whilst Pond-newts (*Triton vulgaris*) are found in every pond and even in every pool of rain-water, and larger sheets of water shelter the crested newt (*Triton cristatus*), the Mountain-newt exercises a more stringent, indeed, a quite specialised choice in the type of its abode. It prefers—at least in the neighbourhood of Vienna—larger ponds with clear water, rich in Algæ and other water-plants, with smooth light-reflecting bottom, especially a bottom of flint pebbles. It is not to be found in the meadow pools of the deeper valleys, but is abundant in the streams and pools of the hills and mountains of the “Wienerwald.” It is perhaps permissible to attribute to this preference for a clear well-illuminated medium, the colour of the Mountain-newt—undoubtedly the most beautiful to be found amongst the members of its family—and the sexual display which is the most accentuated found amongst Newts. The stimulus which provokes this display, as will appear from the observations about to be described, is for the most part an optical one.

The sexual life of our native Urodela constitutes a unique phenomenon in the Animal Kingdom, for whilst the fertilisation is internal, nevertheless there is no copulatory act.

The spermatozoa are deposited by the male in an elaborately formed packet which is taken up by the female into her cloaca. The causal nexus is represented by the sexual display. The male creeps slowly towards the female or approaches her in short quick jumps, touches her body with his snout, then

\* Translated from the German by the Communicator.

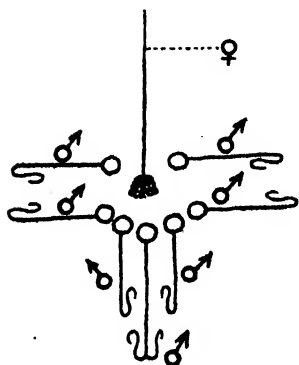
retires a short distance and executes the tail-movement. I shall not take into consideration teleological and especially anthropomorphic and artificial explanations. Whether the male by touching with his snout the female's tail, cloaca and snout, smells her, as Zeller (1890) assumes, or whether he by these methods strives to excite the female and make her receptive, as is usually stated in popular literature, or whether, as appears probable to me, these actions take place accidentally without special purpose during sexual excitement, I will not attempt to decide. The tail-movement consists in the following action. The male bends his tail in hook-like fashion forwards, then he executes vibratory movements with the tail and so brushes his sides (with a pulse of water ?—E.W.M.). A direct contact between the tail and the trunk occurs rarely. Whilst the blows of the tail are being executed, the animal does not shift his place ; at most there is a slight backward movement which, however, is not caused by the tail-movement. His body is thrown into a slight quivering movement, and is occasionally erected into an upright position so that the animal even stands on its toes. During the whole process the cloaca is swollen and the sides of the opening slightly divaricated. Only immediately before the deposition of the spermatophore does the cloaca open to its full extent, and is apparently presented in this condition to the female. By this time the peculiar tail-movements have ceased. The male places himself in such a position before the female that he touches her head with his cloaca. If the female, which is attentively watching him, moves away, he follows her without looking round so that the immediate proximity of his cloaca to her head is maintained. His tail is bent to such an extent that it is perpendicular to the axis of the body. A slight quivering movement of the tail has succeeded to the vigorous blows.

If, however, the female moves far away the male turns and follows her. The blows of the tail begin anew : after hours and occasionally even days of this display the spermatophore is deposited ; the female then creeps over it and takes it up into her cloaca. The present work deals principally with the blows of the tail. It is the object of the research to submit the nature of these movements, their causes, and their relation to the female to experimental analysis. The next three sections are devoted exclusively to the description of the experiments ; and so I may here relate some of my own observations which may throw light on our problem.

The bending of the tail may take place to the right as well as to the left ; this, however, does not occur irregularly. Briefly, the male always bends his tail towards the *side on which he sees the eyes of the female* ; and, indeed, this

occurs with what might be termed geometrical accuracy. This is better shown by the subjoined diagram than by words.

All the positions shown in it I have repeatedly observed, and in every case



I could confirm this rule. Further observations will be required to show whether the rule holds with other Urodeles and whether exceptions to it can ever occur in the case of the Mountain-newt.

It should be mentioned that Newts occasionally "brush" other objects with their tails. Fatio (1879) and Gasco (1881) observed the deposition of the spermatophore by isolated males, and also by males shut up with other members of their own sex. Wolterstorff relates (1922) that Zeller saw a Triton "brushing" an

earthworm. But this was probably a mere tail "wagging" which occasionally occurs in an isolated male, when anything accidentally in the aquarium appears like an object. For the typical tail-strokes only appear without a female on two conditions: first, when a male is isolated for a long time, and in this case the side towards which the stroke is made varies; and secondly, when a great number of specimens are put into a small vessel. Not infrequently I observed that a male *T. alpestris* "paid court" to another male inside the collecting can in which the day's catch was put whilst being brought to the laboratory.

We may remark in passing that there is no difference between the "love-play" as carried out in pools and in the aquarium, i.e., in the wild state as against laboratory conditions. The Mountain-newts are more easily accustomed to captivity than other Newts, indeed they make themselves at home in the collecting can. These observations are not only based on experience in the laboratory, they are also derived from animals living in freedom. Thanks to the clearness of the water in which they live, I have been able to observe Newts mating in pools. The "love-play" occurred exactly as in an improvised aquarium. In this way I could confirm the rule of the relation of the side towards which the tail-stroke is made to the eyes of the females on animals observed in the open.

The problems which the sexual life of the Mountain-newt raises are obvious. The question of the necessity of an orgasm in both sexes in order to render fertilisation possible is at once defined. The male carries out the tail-strokes

before the female, deposits the sperm, and the female picks it up. What are the causes of the various stages, how are they initiated and in what causal relation to each other do they stand?

A complete explanation is, unfortunately, not afforded by the experiments so far carried out. With the exception of the older series noted under Section III, they have all been made outside the limits of the mating period. This circumstance is, however, to some extent advantageous, in that the animals do not begin the tail-stroke spontaneously without the stimulus of experiment, as is uniformly indicated by the controls. The experiments are consequently pure—and in this respect free from objections.

## II.—*The Experimental Production of the Tail Movements in Isolated Males Outside the Limits of the Breeding Season.*

The experiments were made from the middle of December to the end of January. The aquaria were placed on the window sill of a room, the temperature of which was about 15°. Each animal was placed in a separate vessel; plants and special material for the bottom were omitted as they might have had a disturbing influence. The newts had been in the (Biological) Institute since late summer, and had been kept in a big roomy aquarium in which for the most part they collected in groups on bits of bark which floated in the aquarium. They had not assumed their terrestrial "dress" but there was no trace of the formation of the nuptial colours and appearance, that is, of the broad tail with its silvery streaks. I never observed "love-play" during the winter in the large aquarium.

The tail-stroke was experimentally produced by table-salt. A dose of 2 grammes was added daily to the vessel which held 2 litres, so that each day an additional concentration of 1 gramme per litre was attained. At first, the salt was simply sprinkled in from the hand, later about the fifth day, when the reaction had begun it was added by means of a moistened camel's hair brush. In a few days the sojourn in the salt solution produced the full development of the nuptial dress. The skin colour became slate blue, the fin streaked with yellow and black more distinct, the tail broader and its silver streaks more prominent. The cloaca swelled up and became ridged. The tail-stroke, however, was not produced even when a female was added. To accomplish this it is necessary to cause a rain of salt to fall on a male which is already in the salt solution. The brush loaded with salt is held in the water over the animal and shaken so that the undissolved salt grains fall on the animal's back. At first, headlong flight results from this besprinkling.

The feet are pressed against the sides and powerful strokes of the tail bring the animal out of its painful position. If the treatment with salt is repeated frequently every day till the daily dose of 2 grammes is used up, the animal becomes accustomed to it, flight becomes less rapid and panic-stricken and finally ceases. In its place another reaction appears, viz., the tail-stroke.

The male, as often happens when in the presence of the female, goes several steps backwards, bends the tail like a hook towards one side and wags it till the salt is dissolved. The tail stroke is sometimes replaced by a quivering movement of the tip of the tail. The reaction is most beautifully produced when the region of the head is sprinkled with salt. The direction of the tail-stroke once initiated persists. The distinctness of the reaction, and the time when it begins varies with different individuals. The experiment fails when it is too often repeated in one day, but succeeds again after a considerable interval of rest. After two weeks' sojourn in the solution, the experiment loses in promptness and distinctness even when the daily dose is moderate. Leaving out these factors, the tail-stroke, especially at first, can be obtained promptly and with absolute distinctness.

Two control experiments were necessary to determine the active factors more exactly, viz., sprinkling with salt in clean water free from salt, and prolonged culture in salt solution of constant strength. In neither experiment was the tail-stroke ever seen.

We may record the observation that if Newts on dry land are sprinkled with salt, they perish in a few minutes, with the appearance of terrible cramp and paralysis. This result cannot be due to the mere interruption of skin respiration, for rubbing the animals with ashes produces no such effects. If, however, the Newts are at once replaced in water, death can be prevented.

The sprinkling of the salt in the water is carried out either by rubbing it between the fingers and letting it fall slowly on the head of the animal, or a moistened brush is rubbed in the salt and plunged in the water. If the second method is employed for some time with the same animal, then finally only the immersion of the brush, *without adhering salt*, is necessary to initiate the tail-stroke. By gently moving the brush to and fro the attention of the animal is aroused and it reacts more quickly. It is true the brush must from time to time be dipped in salt.

It is, of course, to be understood that in the experiment with the brush alone, it is carefully cleaned and all remains of salt removed.

By leading an electric current through the water, the tail-stroke cannot be initiated, but the bending of the tail and the maximal opening of the cloaca

results. A slight opening of the cloaca was observed to accompany the tail-stroke when this was produced by salt.

### III.—*Artificial Production of the Tail-Stroke in Isolated Females.*

The arrangements for the experiment were similar to those adopted in the case of males. Females isolated and exposed to normal conditions never produced the tail-stroke, but nevertheless I have seen the production of the tail-stroke characteristic and distinct, occurring in females kept in salt solution. It did not take place as in males as a consequence of the animals being besprinkled with salt, but occurred apparently by chance (or rather without recognisable cause). It most frequently occurred in sunshine when the water was rather warm. It resembled the tail-stroke of the male in every detail.

In order to obtain a similar response to that shown by the male to a camel's hair brush, I adopted the following method. Whenever I saw a female executing the stroke, I at once held a brush before her eyes. For the first few times the female interrupts her performance and takes to flight. But the female becomes accustomed to the strange sight much more quickly than the male. This experiment was tried for a week on five females and I was rewarded for my perseverance. The sight of the camel's hair brush started the tail-stroke in two of the females. As with males, it is necessary continually to freshen up the association (or "conditioned reflex"). When, by chance, the tail-stroke is observed, the brush must be at once presented to the animal: then the reaction to the brush continues.

The passage of an electric current through the water does not produce, as it does with most males, a bending of the tail, but rather a rolling-up of the tail towards the inner side; the cloaca does not open.

### IV.—*The Reception of the Spermatophore by the Female.*

Is the reception of the spermatophore by the female causally determined by the previous sexual display ("love-play")?

To answer this question, I carried out a simple experiment. (In order to avoid misunderstanding and misinterpretation I should like to emphasise that by the word *a*, not a single experiment but frequently repeated experiments of the same kind are meant.)

In early summer when the mating impulse is at its height, and when males and females, if placed together in the same vessel, invariably carry out the "love-play," is the best time for the experiment, which was as follows:—

In an aquarium was placed a spermatophore that had just been deposited



In this vessel a female which had become used to the touch of the experimenter was also placed. This female crept about all over the aquarium—incidentally over the spermatophore—but did not take it up. The case was quite different with a female to which a male had just made his “love-play.” Such a female placed in the aquarium approached the spermatophore directly and picked it up into her cloaca. The previous “love-play” is, therefore, a necessary condition for the reception of the spermatophore; the tail-stroke is an element in the causal chain leading to fertilisation.

#### V.—*Concluding Remarks.*

When we attempt to find a theory which will account for the events which have just been described, the most urgent question is to determine how the salt acts in stimulating the animal to begin its tail-stroke.

The female might excrete salt which acted as an aphrodisiac on the male and incited him to begin the tail-stroke. Chemical influences acting as an aphrodisiac would not constitute an isolated phenomenon. By means of the (chemical) olfactory sense the male of most mammalian species recognises the female. The secretions of the female are thus capable of acting as an aphrodisiac on the male.

In our experiment the stimulus of the NaCl might perhaps have sufficed to induce the tail-stroke in the male.

A second possibility is that NaCl, like other injurious factors, tended to bring the sexual impulses to full development. The sudden growth of the sexual impulse after the starvation period of the winter sleep, the frequent absence of sexual development when the sleep is prevented and generous feeding given, certainly constitute evidence for this view. Indeed, Kammerer (1907) states “that sickly animals exhibit a particularly strong sexual impulse, as if they felt it necessary to hasten with the arrangements for the increase of the species.” That salt is an extremely injurious factor for Newts is evident from the fact that when it is rubbed into them when they are on dry land they quickly perish. Without excluding these explanations, I am inclined to favour a third alternative, viz., that sodium chloride acts as a sensitiser. The animals become more sensitive and excitable by being kept in a salt solution. A further stimulus, the renewed addition of salt, starts the reaction, which fails to take place in animals kept in tap-water and which, therefore, have not been sensitised. Perhaps we may find here an application of the Weberian law, since the reaction does not take place in higher concentrations of salt solution.

The experiment with the camel's hair brush strongly suggests the conclusion that the secondary sexual characters of the partner, considered as sexual stimuli, are "conditioned reflexes."

If the orgasm is always accompanied by the sight of particular parts of the body or the olfactory perception of certain substances, then both impressions are so connected together that—still considering the case we have chosen—the sight of portions of the body can favour and hasten the beginning of the orgasm. How far sexual inversion in mankind (Fetishism, etc.) owes its origin to such a "conditioned reflex," and if by taking into account these results it could be cured, we cannot now discuss. This question may be reserved for a subsequent work on the biological roots of human perversion.

The part played by the camel hair brush in the brief laboratory experiment may, in the phylogenetic history of the race, have been assumed by the secondary sexual characters of the other sex which have later become specific stimuli. Whether we have to do with a psychical or merely a sensory association is very difficult to decide. In any case the objection made against the theory of sexual selection, that it presupposes an æsthetic sentiment, is no longer valid. That, further, not only bodily characters (Tandler, Frost) but also functional peculiarities are characteristic of species, is proved by the possibility of producing the tail-stroke in the Triton female.

The "love-play" of the Newts, therefore, appears to me—I might almost say—puzzling, because in this case the same cause does not, as in most other cases, lead to the expulsion of the sexual cells and to fertilisation. In this case we have neither coitus nor copulation, and yet fertilisation takes place; it is true, not with the inevitability or security provided by these two latter forms of sexual union. The object of the present work is to explain the probable nature of this causal mechanism.

#### *Summary of Results.*

(1) The Mountain-newt (*Triton alpestris*) has like other Newts internal fertilisation without sexual union. The spermatophore is deposited by the male and afterwards sucked up by the female into her cloaca. This act is preceded by "love-play." The male approaches the female, bends his tail forwards in a hook-like manner and executes quick waving movements with it. The tail is always turned toward the side on which the eyes of the female are situated.

(2) If males of the Mountain-newt, whilst kept in a salt solution, are sprinkled with salt, they execute the tail-stroke. Later, it is sufficient to plunge into

the solution the brush with which previously the salt had been taken up, in order to initiate the tail-stroke.

(3) If females are kept in salt solution they also execute the tail-stroke, but this is not initiated by a definite stimulus. However, if the camel's hair brush is frequently held before the eyes of a female when she is executing the stroke, then subsequently she will react to the presentation of the brush by initiating the tail-stroke.

(4) Previous "love-play" by the male is a necessary pre-requisite to the taking up of the spermatophore by the female.

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### *Studies in Amphibian Colour Change.—II.*

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(Communicated by Prof. E. W. MACBRIDE, F.R.S.)

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#### *Introduction.*

The author, in a previous paper, has dealt with the normal reactions of *Rana temporaria* to various environmental factors. It is hoped here to deal with the method by which these factors influence the mechanism which, in turn, produces the characteristic responses in the melanophores.

The effect of the background has been shown to be as follows :—

Light background tends to produce pallor ;

Dark background tends to produce darkening.

Parker has shown that the frog makes heliotropic movements when it is blinded, and postulates that it possesses some receptor organ in the skin sensitive

to light. It may be suggested that the frog only perceives the stimulus of heat. At the present state of the investigations it is impossible to distinguish between the two. For the frog shows a definite response to heat stimuli similar to the response to light.

Experiments were carried out to show whether the frog would respond to background when blinded. For this purpose it was not necessary to repeat all the combinations of factors employed in the normal reactions. Many of these would not show any difference from the normal as the other factors tend to produce the same result as the background, so that if the factor of background were eliminated by blinding the frog the response would be in the same direction as before and thus not be plainly demonstrable. Those conditions found to be most suitable for these experiments are :—

$$\text{Ex. 1. } \begin{cases} \text{Mt} + \text{L} + \text{Lb} + \text{W} \\ \text{Mt} + \text{L} + \text{Db} + \text{D} \end{cases}$$

$$\text{Ex. 2. } \begin{cases} \text{Lt} + \text{L} + \text{Lb} + \text{D} \\ \text{Lt} + \text{L} + \text{Db} + \text{D}^* \end{cases}$$

In Ex. 1 the normal results are close together, each set of factors containing two opposing stimuli. There is, therefore, a delicate balance established, so that, if one of the opposing factors be removed in each of the sets of factors then a definite swing over in one direction or another should occur. In this case the results, if blinding the frog has any effect, should be separated widely. In Ex. 2 the normal results are wide apart, this difference being due to difference of background in the two sets of conditions. Again, if the background is eliminated by the blinding of the frog, the results of these two sets of conditions should be identical in all respects.

Hogben has stated that neither pilocarpine nor atropine has any effect on the colour responses of the frog. A loophole presents itself, however, for these experiments were carried out under conditions which, although suitable for producing maximum of pallor or darkening as required, were not suitable for showing any small change; due to the elimination of one factor alone for instance. Kahn, moreover, has found that pilocarpine causes expansion of the melanophores when the frogs are kept moist—one of the conditions already mentioned as capable of showing small changes.

Experiments have been carried out with injections of pilocarpine into normal, blinded, and other frogs which have had the cutaneous nerves cut.

These experiments have been carried out in Prof. McBride's laboratory

\* Mt = medium temperature (15°–17° C.); Lt = low temperature (0°–2° C.); Lb = light background; Db = dark background; W = in water; D = dry.

at the Imperial College of Science. The author's thanks are due to Prof. McBride for advice and facilities rendered.

### *Adrenalin.*

Lieben (1906) found that adrenalin caused a contraction of the melanophores of *Rana temporaria*. This has suggested that adrenalin may be connected with the colour changes in the normal reactions. Hogben and Winton (2) have suggested that these colour changes may be due to a balance of post-pituitary and adrenal secretions. They doubt, however, if this is possible owing to the rapid decomposition of adrenalin which must take place in the blood.

Experiments have been carried out to determine whether there is any connection between colour and adrenalin secretion in the frog:—

1. Five frogs were placed on white background, dry and in the light at medium temperatures (15°–17° C.). After two hours they were extremely pale. They were then killed and skin removed from the back of each and fixed in Bouin. The kidneys were also removed and fixed in Osmic vapour for 20 minutes; taken into 50 per cent. alc.; upgraded into xylol and embedded (Cramer's method).

2. Five frogs were placed on black background, in water and at medium temperatures. After two hours they had become dark and were treated in the same way as the pale ones.

The following is the result of examination of the skin and adrenal tissues:—

Table I.

Pale Series.			Dark Series.		
No.	Skin.	Adrenals.	No.	Skin.	Adrenals.
P. 1.	–1.5	Medium secretion.	D. 1.	+1	Medium secretion.
P. 2.	–1.5	A little secretion.	D. 2.	+2	Large secretion.
P. 3.	–1.5	A little secretion.	D. 3.	+0.5	Medium secretion.
P. 4.	–1.5	Medium secretion.	D. 4.	+0.5	A little secretion.
P. 5.	–2	No secretion.	D. 5.	+0.5	Large secretion.

The operation of removing the skin and kidneys after killing the animals did not take more than three minutes in any case, frequently less. It will be observed that while the darker frogs show a greater secretion on the

whole than the pale ones there is a considerable divergence in the results among those of the same class. While it may be argued that the increased adrenalin secretion is balanced by a greatly increased pituitary secretion (and it undoubtedly is) an anomalous case arises where no secretion is found, or where so little that it must be negligible in stimulating sympathetic nerves, the frog being pale. It is, therefore, unlikely that adrenalin takes any significant part in the colour changes of the frog.

#### *Reactions of the Xantholeucophores.*

The reactions of the xantholeucophores to normal environmental stimuli at first sight appear to be discordant. Their true value is seen, however, when they are compared with the reactions of the melanophores in each individual. It is unnecessary to tabulate the results which may be briefly stated thus:—Wherever the melanophores are expanded the xantholeucophores are contracted. Where the melanophores are contracted the xantholeucophores may be either contracted or expanded.

The former result is well illustrated in one set of conditions in which one out of seven frogs had stellate melanophores, all the rest being in disperse phase. This one alone had slightly expanded xantholeucophores, the rest having extremely contracted ones.

The action of pituitary secretion is well known in that it contracts the xantholeucophores while it expands the melanophores. The identity of the two results—i.e., action of post-pituitary secretion and the action of environmental stimuli which produce expansion of the melanophores—confirms the theory that the post-pituitary autocoid plays an important part in the colour changes of the frog.

#### *Experiments on Blinded Frogs.*

The frogs were blinded by sectioning the optic nerve through the roof of the mouth. Ether and eucaïne were used as anæsthetics. The frogs were not used for experiments until at least 24 hours afterwards. The system of obtaining numerical results is the same as that employed in the normal reactions.\*

\* These figures are arrived at thus:—A series of photographs of five equally spaced phases of expansion of the dermal melanophores is used as a standard. Numbers from -2 to +2 are given to denote these phases and each preparation of skin when examined is given a number to denote its position on the scale. The numbers are of course purely arbitrary and depend on the rate of expansion being constant. The advantage of this system over that of descriptive words is that it permits an average to be struck with greater accuracy.

*Experiment 1.*

(Blinded)		1	2	3	4	5	6			(Normal)	
								Av.	P.E.	Av.	P.E.
Mt+L+Lb+W		+1	+1	+2	+1.5	+1	+2	+1.4	±0.13	-1.0	±0.24
Mt+L+Db+D		-1.5	-1.5	0	-1	-1	0	-0.8	±0.19	-1.3	±0.22
Differences								-2.2		-0.3	

*Experiment 2.*

(Blinded)		1	2	3	4	5	6			(Normal)	
								Av.	P.E.	Av.	P.E.
Lt+L+Lb+D		0	-1.5	0	-0.5	-1.5	-1	-0.8	±0.19	-1.1	±0.10
Lt+L+Db+D		-1	0	0	+0.5	-1.5	+1	-0.2	±0.25	-0.1	±0.17
Differences								-0.6		-1.2	

From the first experiment it will be observed that the elimination of all stimuli which are normally received through the eye has a profound effect on the colour responses. In the second half of this experiment it will be seen that the frogs are darker than in the normal controls, whereas it would be expected that by the elimination of the black background the frogs would be lighter. Allowances for this darkening effect must be made in all these experiments. The differences are, therefore, given in all cases.

In the second experiment the expected identity of result is not completely realised. It may be argued that the difference is not sufficiently great for any emphasis to be laid upon it. At the same time it must be recognised that the difference is half that obtained in the normal reactions and is 15 per cent. of the total possible. Moreover, the probable errors show that there is a distinct difference in the results obtained. It is, therefore, evident that there are stimuli received from the skin, as well as from the eye.

It has been noted above that the blinded animals are, on the whole, darker than the normal. This may be due to the operation, but after the lapse of time always allowed it is probable that this will have passed off. It would, therefore, seem that the stimuli from the eye are of an inhibitory nature.

The situation is then as follows:—Light background causes a stimulation of the nerves in the eye which in turn inhibits the secretion of the pituitary. The stimuli from the skin seem to be of a similar nature.

The method of stimulation of the pituitary (posterior lobe) is still obscure. The nerve supply to the posterior lobe is uncertain, the following sources being claimed:—\*1. Central (infundibular); 2. Sympathetic.

\* The author wishes to express his thanks to the following for kindly giving him the information required:—Sir E. S. Schafer, Sir F. W. Mott, Profs. Starling, Elliott-Smith and Herring.

In order to throw some light, if possible, on this problem the experiments with pilocarpine and atropine were undertaken.

*Pilocarpine Reactions.*

The following experiments were carried out :—

1. Six normal frogs which had previously been placed in water on a white background for 12 hours were injected with 2 mgms. pilocarpine. They were subsequently replaced on their white background and in water at medium temperatures. No darkening occurred during the period of observation (3 hours).

2. Six normal frogs under exactly similar conditions as (1) were injected with 8 mgms. pilocarpine and observed for 6 hours. Slight darkening occurred but it was not very obvious.

3. Six normal frogs as before were injected with 15 mgms. pilocarpine. After 6 hours three of these were partially paralysed and showed darkening of the skin ; the others also showed some darkening but not to the same extent.

4. Six normal frogs as before were injected with 12 mgms. pilocarpine. After 3 hours all showed marked darkening and slight paralysis. These frogs were selected as being of a uniform colour and size ; and had been under observation for 2 days previously. The slightly different results between this and the previous experiment are due to the smaller size of the frogs employed in this last experiment.

In the case of all these four experiments the frogs were selected for their uniformity of colour. The latter only being selected for size as well, on account of the influence of this factor on the reaction to pilocarpine. Preparations were made of the skin on the last experiment. The result is given in Table II.

5. Six frogs were blinded and after 24 hours placed on black background, dry at medium temperatures for 24 hours longer. They were then injected with 15 mgms. pilocarpine. One died during the experiment. No paralysis shown by them. Slight darkening.

6. Five frogs were blinded and two days after the cutaneous nerves sectioned. They were then placed in water on a white background and 2 days later injected with 20 mgms. pilocarpine. After 3 hours preparations were made. Two were fairly dark, while the rest remained pale. The results are given numerically in Table III. The completeness of the operation was also noticed and is tabulated as well.



Table II.—Normal Frogs Injected with Pilocarpine.\*

No.	Skin.	No.	Skin.
1.	+0.5	5.	+0.5
2.	0	6.	+1
3.	+1	7.	0
4.	+1	8.	+1

Table III.—Frogs with Cutaneous Nerves Sectioned Injected with Pilocarpine.

No.	Skin.	Condition of cutaneous nerves.
1.	+1	Few lateral nerves remained uncut.
2.	+1	Several lateral and one dorsal nerves uncut.
3.	-1.5	All out except a few abdominally.
4.	-1	All out.
5.	-0.5	All out.

All injections were made intraperitoneally and controls injected with Ringer. The latter had no effect.

From Experiments 1-5 it must be concluded that pilocarpine causes a darkening of the frog. From Experiment 6, however, it is obvious that it causes this darkening, not by stimulating the pituitary to secrete, but by some action on the cutaneous nerves. The position will become clearer when the action of atropine is taken into account.

Table IV.—9 mgms. Atropine Injected into 5 Normal Frogs.

No.	Skin.	Condition of frog.
1.	+1	Paralysed.
2.	+1	Paralysed.
3.	+1.5	Paralysed.
4.	-0	Slightly paralysed.
5.	-1.5	No paralysis.

\* Nos. 1-3 are from Experiment 3, Nos. 4-8 from Experiment 4. The figures under the heading "skin" represent the condition of the melanophores as explained before.

*Atropine Reactions.*

The following experiments were carried out :—

1. Three frogs (normal) injected with 1 mgm. atropine and replaced on white background in water at medium temperatures. No change in colour during 3 hours was observed.

2. Three frogs (normal) injected with 3 mgms. and replaced on white background in water at medium temperatures. No change in colour.

3. Five pale normal frogs placed in water on white background at medium temperature. Marked darkening within 2 hours after injection of 9 mgms. atropine. Numerical results are shown in Table IV.

4. Five blinded frogs in water on white background and at medium temperature were injected with 9 mgms. atropine. One of the frogs died soon after the injection. Although fairly dark at the beginning of the experiment they became darker in about 1 hour's time. After this they started to become pale in patches, these patches enlarging until the whole animal was uniform as at the commencement of the experiment.

5. One blinded frog with cutaneous nerves cut under the same conditions as the above was injected with 9 mgms. atropine. Darkening ensued but was not followed by a subsequent blotched paling.

All the injections were made intraperitoneally and controls injected with Ringer remained in their respective conditions.

It would seem then that atropine has two actions on the colour. The first is that of darkening, which is independent of the skin; the second that of causing pallor, which is the reverse of the pilocarpine reaction, and which acts on the same cutaneous nerves as pilocarpine.

*Conclusions.*

From the facts that the blinded frogs are all darker than the normal ones in Experiment 1, and that those on the white background and in water were darkest, it may be deduced that the stimuli from the eye are inhibitory in nature and that paralysis of these nerves would cause a darkening of the skin under suitable conditions.

Now atropine paralyzes the parasympathetic nerves and most of the results obtained by stimulating these nerves can also be had by means of pilocarpine. Their action is, however, by no means confined to the parasympathetic nerves, as frequently they affect sympathetic fibres. Moreover, reversal of these actions is known in certain cases.

But if all this is borne in mind it may be stated :—That the parallelism of results obtained when the optic nerve is sectioned on the one hand, and the injection of atropine on the other, suggests that the stimuli received by the eye affect the posterior lobe of the pituitary (causing inhibition of secretion) by means of the autonomic (possibly parasympathetic) nervous system.

The opposing actions of pilocarpine and atropine which are eliminated by degenerative section of the cutaneous nerves are capable of more than one interpretation. Hogben and Winton have shown that if a sympathetic control exists it causes contraction of the melanophores when stimulated and does not take part in the normal responses. If this then be taken as a basis it must be postulated that the pilocarpine and atropine are acting on sympathetic fibres and are, moreover, having a reversal of effect.

On the other hand, pilocarpine is known to have a peripheral vasomotor action causing dilation of the blood-vessels in certain cases (Langley). Amsler and Pick, however, did not find that it affected the perfused vessels of the frog. An increased flow of blood carrying the pituitary autocoid might cause the expansion of the melanophores.

The result is peculiar and no really satisfactory explanation is at hand.

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*The Immediate Changes Observed in Tissue Cells after Exposure to Soft X-Rays while Growing in Vitro.*

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[PLATES 20 AND 21.]

The experiments described in the following paper were carried out in order to study the immediate changes seen in tissue cells after exposure to soft X-rays while growing *in vitro*.

The tissue from which the cultures were grown was obtained from the choroid of chick embryos of 6 to 7 days' incubation.

One of us (S.) had previously studied cultures *in vitro* of this tissue, grown under both favourable and unfavourable conditions, and was therefore in a position to recognise with some degree of confidence changes in the growth and structure of the cells. The tissue cultures were made in chick plasma and chick embryo extract on a No. 1 soda glass coverslip inverted over a hollow ground slide of soda glass sealed with paraffin, and incubated at 39° C. Twenty-four hour old sub-cultures were used, and before exposure to X-rays they were examined to see if the growth was good and the cells in mitosis were plentiful. The cultures were exposed to soft rays filtered through 2 mm. of cardboard. The alternative spark gap was 8 cm. with a rectifying spark gap of 2.5 cm. and a water resistance in series with the tube. The reason for this arrangement was that it was found impossible with the apparatus available to suppress the inverse current through the tube with the rectifying spark gap only. The distance of the cultures from the anticathode was 24 cm., and while running the resistances were continually adjusted so that occasional sparking was taking place across the equivalent spark gap. As far as possible the current was kept at 1 ma. ; at times it rose a little above this, but very rarely exceeded 1.2 ma.

To keep the cells at incubation temperature a double-walled wooden box packed with shavings between the walls was used. The internal dimensions of the chamber were 32 cm. in length, 26 cm. in width, 29 cm. in depth. A wooden platform was fitted on which four cultures could be placed. The top of this

platform was 21 cm. from the bottom of the chamber. The open top of the box was covered with cardboard 2 mm. in thickness, with a glass window at one side, through which a thermometer laid on the platform beside the cultures could be watched. Two electric lamps were placed on the bottom of the box, and by occasionally switching one of these on or off the temperature was kept in the neighbourhood of  $37.5^{\circ}\text{C}.$ ; it was not allowed to fall below  $36.5^{\circ}\text{C}.$  or rise above  $39^{\circ}\text{C}.$

A 12-in. coil was used, with a mercury jet break with gas dielectric, and a tungsten target gas tube. The dose given was measured by time, as it was impossible to get pastille readings corresponding to the small increases of dose given in the various experiments.\*

Four cultures were exposed to the rays in each experiment, two were stained immediately, and two, for reasons given later, were returned to the incubator for 80 minutes before staining. The cultures were fixed in acetic alcohol and stained with iron hæmatoxylin.

The cultures were exposed to soft X-rays for varying periods and the experiments are best described as two separate series :—

- (1) Cultures fixed immediately after varying periods of exposure.
- (2) Cultures fixed after 80 minutes' incubation subsequent to varying periods of exposure.

*Cultures fixed immediately after varying periods of exposure.*

Cultures exposed for 5, 10 and 15 minutes respectively show in every specimen numerous cells in prophase and various phases of mitosis, and in these cells no recognisable changes from the normal can be distinguished. The fully formed cells appear normal.

Cultures exposed for 20 minutes show a decrease in the number of cells in prophase, but cells in other phases of mitosis are plentiful. No abnormalities can be recognised either in cells in prophase or other phases of mitosis. The fully formed cells appear normal.

Cultures exposed for 25 minutes show very few cells in prophase, and the number of cells in other phases of mitosis is distinctly less than in the controls. No abnormalities of the chromosomes can be recognised. The fully formed cells appear normal.

Cultures exposed for 30 minutes show only two or three cells in prophase and a definite

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\* After the experiments recorded were finished an Ionto-quantimeter was obtained at the suggestion of Dr. Hopwood in order to carry out further observations. The Ionto-quantimeter was one designed and calibrated by Prof. Friedrich, of Freiburg, Baden. By the use of this instrument the "dose" or "exposure" received by an object placed in an X-ray beam is measured by absolute units. The units employed, designated by "1 e," is that amount of energy of beam which will produce in 1 c.c. of air under normal conditions an amount of ionisation that is equivalent to a change of one electrostatic unit of quantity of electricity in the electrometer. In these experiments the incident beam was of such an intensity that 1 e was given per minute.

decrease in the number of cells in other phases of mitosis. No abnormalities of the chromosomes can be recognised. The fully formed cells appear normal.

Cultures exposed for 35 minutes show only one or two cells in prophase or in other phases of mitosis. Some of the cells in mitosis show granular changes in the chromosomes during metaphase and anaphase. The fully formed cells appear normal.

Cultures exposed for 40 minutes. In many cultures there are no cells in prophase, in other cultures one or two cells in prophase are seen. The majority of cultures show only one or two cells in mitosis, in some of which the chromosomes show granular changes during metaphase and anaphase. The fully formed cells appear normal.

Cultures exposed for 45 minutes. In the majority of cultures there are no cells in prophase, but in a few one or two are found. There are one or two cells in other phases of mitosis in most cultures. The chromosomes show granular changes and fragmentation during metaphase and anaphase. The fully formed cells show no definite change.

Cultures exposed for 50 minutes. In the majority of cultures there are no cells in prophase and only one or two cells in other phases of mitosis, which show granular changes and fragmentation of the chromosomes with lag in division. The fully formed cells show no definite changes.

Cultures exposed for 55 minutes. In the majority of the cultures there are no cells in prophase, but in most cultures there are one or two cells in abnormal mitosis, which show granular changes and fragmentation of some or all of the chromosomes and also a definite lag in division. The fully formed cells show no definite change.

Cultures exposed for 60 minutes. In the majority of cultures there are no cells in prophase, and many cultures show no cells in mitosis. In a few, however, there are one or two cells in abnormal mitosis showing marked granular changes and fragmentation of the chromosomes and a definite lag in division. The nuclei and nucleoli of some of the fully formed cells at the outer zone of the culture are enlarged and the nucleoli show decrease in density and unequal staining. In a few specimens one or two early breaking-down cells are seen.

Cultures exposed for 75 minutes. In the majority of the cultures no cells in mitosis are found, but in a few there are one or two cells in abnormal mitosis showing granular change and fragmentation of the chromosomes and lag in division. In some of the fully formed cells at the outer zone of the culture there is a definite increase in the size of the nuclei and nucleoli. Many of the nucleoli show marked change, they are enlarged and appear more granular, but less dense than normally. The majority of the fully formed cells show no change. In a few cultures early breaking-down cells are seen.

Cultures exposed for 85 minutes. In the majority of cultures there are no cells in prophase or in other phases of mitosis. In a few specimens there are one or two cells in abnormal mitosis showing granular change and fragmentation of the chromosomes with lag in division. Many of the cells at the periphery of the growth show increase in size of the nuclei and nucleoli; some of the nucleoli are enlarged and show a decrease in density and appear granular. The area occupied by the cytoplasm of some of these cells is also increased and the cytoplasm itself appears more granular. The majority of the fully formed cells show no change. Early breaking-down cells are present in most cultures.

Cultures exposed for 120 minutes. In the majority of cultures there are no cells in prophase or other phases of mitosis. In a few specimens there are one or two cells in abnormal mitosis showing granular change and fragmentation of the chromosomes and lag in division. There are definite changes in the nuclei and nucleoli of many of the fully formed cells at the outer zone of the culture. The nuclei of these cells are enlarged and the

nucleoli in many of the enlarged nuclei are also much altered, being increased in size and showing alteration in density and unequal staining. Some nucleoli show deeply staining coarse granules, held together by coarse threads. The cytoplasm of these cells is increased and shows granular changes. The majority of the fully formed cells show no change. Breaking-down cells in various stages are present in all cultures.

In the first series of experiments the earliest change noticed is that, after an exposure of 20 minutes, fewer cells in prophase are found, and that after 30 minutes or longer cells in prophase are scarce. Cells in the later phases of mitosis begin to diminish in number after an exposure of 25 minutes, and after 35 minutes or longer only one or two are found.

If not exposed to the rays for longer than 30 minutes the cells already in prophase pass through the various phases of mitosis, the chromosomes show no abnormality and the cells divide normally (figs. 1, 3, 5, 7 and 10). After 35 minutes or longer, however, definite changes may be seen in the chromosomes during the metaphase and during the later phases of mitosis, and these changes become more pronounced as the length of exposure is increased. The first abnormality seen is a granular change in some of the chromosomes at metaphase and anaphase (figs. 2 and 4). As the length of exposure is increased more cells show this abnormality, the granular changes in the chromosomes become more distinct and some of the chromosomes are definitely broken up into small fragments (fig. 6). After exposures of 50 minutes or longer some of the cells in mitosis show a distinct lag in division, both during metaphase and in the passage of some of the chromosomes, or their fragments to the poles of the spindle at anaphase (fig. 8). The lag of the chromosomes during anaphase may persist until telophase is well advanced, so that the two phases become merged into one. In spite of these abnormalities the cell divides and forms two daughter cells, but a lag in the formation of these may be found also (fig. 9). After the longer exposures, however, in some of the daughter cells two or three nuclei may appear (fig. 11) and occasionally marked changes occur in one or both daughter cells, which will be described later under the term of "breaking down cells" (fig. 21).

After exposures of less than 60 minutes no changes are seen in the cells which are in a resting condition (fig. 13), but after an exposure of 60 minutes or longer changes are seen in some of them, especially in those at the outer zone of the growth. The nuclei of such cells are somewhat larger than usual, the nucleoli are increased in size and show irregularity of outline, some stain unequally and appear granular. Changes are also seen in the cytoplasm of the affected cells which is increased in area and appears less dense and more granular (fig. 14).

Marked changes are found in some cells after an exposure of 60 minutes and the number of such cells increases up to a certain point with the length of exposure. These cells, which are recorded as "breaking-down cells," show definite changes in the nucleus and cytoplasm. In many, the first change seen is the appearance of particles of deeply staining chromatin in the nucleus. These may first appear as small angular bodies on the inner side of the nuclear wall (figs. 16 and 17), but these soon become detached and are seen as small bodies lying within the nucleus (fig. 15). At the same time, or soon after, the outline of the cell alters and often becomes irregular, and later the cytoplasm begins to break up (figs. 15 and 17). In other cells the nucleus and cytoplasm appear to disintegrate suddenly and beautiful ray-like structures result (fig. 20). The rays are formed of small pear-shaped masses of cytoplasm, many of which contain larger or smaller droplets derived from the nucleus, in some of these droplets a particle of deeply staining chromatin is enclosed. In other cells the process is more gradual; the outline of the nucleus alters in shape and eventually breaks up into several small bodies, some of which also contain deeply staining particles (fig. 18). The cytoplasm at the same time shows a most irregular outline and fine thread-like processes may also be seen. The broken-down cells vary much in size and shape (figs. 19 and 21) and become disorganised so that only shadows of scattered fragments of the cytoplasm and the nucleus remain (fig. 22), eventually even these disappear and no trace of the cell can be found.

Thus the first series of experiments show that definite changes can be recognised in the cultures after 30 minutes' exposure, and that these changes become more pronounced as the time of exposure is increased; but they do not show if the effect of the X-rays on a cell is immediate, or if there is a latent period before it can be recognised. It was in order to determine this point that two cultures in each experiment were returned to the incubator for 80 minutes before fixation.

This period of 80 minutes was selected as observations already published (Strangeways, 1922) (1) show that this allows ample time for cells to pass through the various phases of mitosis and to complete division under favourable conditions. It is also long enough to show if any lag in division occurs or if changes develop in the fully formed growing cells within a relatively short time after their removal from the influence of the rays.

*Cultures fixed after 80 minutes incubation subsequent to varying periods of exposure.*

Cultures exposed for 5 minutes show that cells in mitosis are already diminishing, but there are still several cells in prophase and other phases of mitosis. In a few there are



granular changes in the chromosomes which is first seen at metaphase. In the fully formed cells no changes can be recognised.

Cultures exposed for 10 minutes show fewer cells in prophase and other phases of mitosis. There are definite granular changes with fragmentation of the chromosomes in some cells during metaphase. In the fully formed cells no changes can be recognised.

Cultures exposed for 15 minutes show only a few cells in prophase and other phases of mitosis. The majority of the chromosomes show granular changes and fragmentation. In some there is a definite lag in the passage of the chromosomes to the poles of the spindle. In the fully formed cells no changes can be recognised.

Cultures exposed for 20 minutes show only one or two cells in prophase and a few cells in other phases of mitosis, with granular changes and fragmentation of the chromosomes. In many of these there is also a definite lag in some of the chromosomes to the poles of the spindle. In a few cells at metaphase clumping of the chromosomes is seen. In the fully formed cells no changes can be recognised.

Cultures exposed for 25 minutes show only one or two cells in prophase. Cells in other phases of mitosis are scarce, most of these show granular changes and fragmentation of the chromosomes and also lag in division. In a few cells at metaphase clumping of the chromosomes is seen. In the fully formed cells no changes can be recognised.

Cultures exposed for 30, 35 and 40 minutes respectively show in the majority of cultures no cells in prophase or other phases of mitosis. In a few cultures one or two cells in abnormal mitosis are present, some of which show either granular changes and fragmentation of the chromosomes with lag during division, or clumping of the chromosomes at metaphase. In the fully formed cells no changes can be recognised. One or two breaking-down cells are seen.

Cultures exposed for 45 minutes. In the majority of the cultures there are no cells in prophase or other phases of mitosis. In a few specimens there are one or two cells in abnormal mitosis, some of which show granular changes and fragmentation of the chromosomes with lag in division or clumping of the chromosomes at metaphase. In some of the fully formed cells at the outer zone, early changes are seen. The nuclei and nucleoli are increased in size and some of the nucleoli stain unequally. In the majority of the fully formed cells no changes can be recognised. A few breaking-down cells are found.

Cultures exposed for 50 and 55 minutes. In the majority no cells in prophase or other phases of mitosis are seen. In a few cultures there are one or two cells in abnormal mitosis which may show either granular changes and fragmentation of the chromosomes with lag in division, or clumping of the chromosomes at metaphase. A large number of cells show changes in the nuclei and nucleoli. In most of the fully formed cells no changes can be recognised. A few early breaking-down cells are seen.

Cultures exposed for 60 minutes. In the majority of the cultures no cells in prophase or other phases of mitosis are found. In a few cultures there are one or two cells in abnormal mitosis which show either granular changes and fragmentation of the chromosomes with lag in division, or clumping of the chromosomes at metaphase. The nuclei and nucleoli of many cells at the edge of the culture show increase in size and some of the nucleoli stain unequally. The cytoplasm of some of these cells is increased in size and shows granular changes. In the majority of the fully formed cells no changes can be recognised. Several breaking-down cells in various stages are present.

Cultures exposed for 75 minutes. In the majority of cultures there are no cells in prophase or other phases of mitosis. In a few cultures there are one or two cells in abnormal mitosis which show either granular changes and fragmentation of the chromosomes with

lag in division, or clumping of the chromosomes at metaphase. A larger number of the fully formed cells show increase in size of the nuclei and nucleoli and many of the nucleoli stain unequally. The cytoplasm of many of these cells is increased in size and shows granular changes. In the majority of the fully formed cells no changes can be recognised. Breaking-down cells in all stages are present.

Cultures exposed for 85 minutes. In the majority of the cultures there are no cells in prophase or other phases of mitosis. In a few cultures there are one or two cells in abnormal mitosis which show either granular changes and fragmentation of the chromosomes with lag in division, or clumping of the chromosomes at metaphase. The number of fully formed cells showing increase in size of the nuclei and nucleoli is larger and many of the nucleoli stain unequally. The cytoplasm of many of these cells occupies a larger area and shows granular changes. In the majority of the fully formed cells no changes can be recognised. Breaking-down cells in all stages are present and in some of these the cytoplasm and nucleus are so broken up that only isolated fragments remain, the rest of the cell appears to have gone into solution in the culture medium.

Cultures exposed for 120 minutes show practically no cells in mitosis, but if mitosis is present the chromosomes are clumped or show granular changes and fragmentation with lag in division. The changes in the nuclei and nucleoli of many of the fully formed cells are marked. In addition to increase in size, some of the nucleoli appear as granules held together by coarse threads. In the majority of the fully formed cells no changes can be recognised. Numerous breaking-down cells in all stages are present, some of these are so broken up that only isolated fragments of the nucleus and cytoplasm remain, part of the cell appears to have gone into solution in the culture medium and some of the broken-down cells have undoubtedly dissolved. This complete solution of the broken-down cells has been confirmed by watching such cells break up and disappear on a warm stage.

In this series an interesting abnormality, consisting of a clumping or agglutination of the chromosomes in some cells at metaphase, is observed (fig. 12). Such cells do not divide, but remain for a time at rest and eventually break up, and undergo disintegration.

In both series of experiments the changes are similar, but a comparison of the cells fixed immediately after irradiation with those returned to the incubator for 80 minutes shows that there is a latent period of about 15 to 20 minutes before the changes in the cells can be recognised.

The changes in the cells described above do not appear to differ from changes found in the cells of cultures grown under the influence of many other abnormal conditions. They may be found in cultures growing in unfavourable medium and in cultures repeatedly sub-cultured if the plasma and tissue extracts are not carefully controlled.

#### *Discussion.*

(1) The second series of experiments show that after 5 minutes' irradiation the development of new dividing cells diminishes though the cells already in mitosis complete their division normally, and after exposure of 20 minutes or

longer the formation of dividing cells practically ceases. It is important to note that if cultures in which mitosis has ceased are returned to the incubator for some hours, cells in mitosis are again found and unless a heavy dose of X-rays has been given they become plentiful, but after the longer exposures the mitosis is abnormal. Attention has been called to the suspension of mitosis after irradiation by Lacassagne and Monod (1922) (2), who exposed to the X-rays a sarcoma in a dog. They state "*la disparition à peu près complète de toutes les figures de division cellulaire*" in the case of sections fixed immediately after irradiation of 5 hours 20 minutes, in which a dose of 20.5 H. Units Bordier Nogier was given. Apolant (1904) (3) also found scanty karyokinesis after irradiation by X-rays.

(2) The granular changes and fragmentation of the chromosomes observed at metaphase and anaphase are noticed in a few cells in the second series of experiments after exposures of only 5 minutes. After 15 minutes' exposure the majority of cells in mitosis show such changes, in addition there is often a definite lag in the passage of some of the chromosomes to the poles of the spindle. With the longer exposures there is often a definite lag in the division of the cell itself, but in spite of these changes many of the cells, if not the majority, pass through the various phases of mitosis and complete division. After the shorter exposures two apparently normal daughter cells are formed, but after a long exposure the daughter cells occasionally show abnormality and may contain two or three nuclei. It is interesting that cells in mitosis showing such abnormality of the chromosomes should be able to pass through the various phases of mitosis and form two daughter cells. Granular changes and fragmentation of the chromosomes have been noted by other workers, Perthes (1904) (4), Koenicke (1906) (5), Clunet (1910) (6), Hertwig (1911) (7), Mottram (1913) (8).

In the second series clumping of the chromosomes at metaphase is seen in some cells after an exposure of 25 minutes, which is more frequent as the length of exposure is increased. Clumping of the chromosomes during metaphase has previously been observed by Apolant (1904) (3).

(3) The increase in size and alteration in structure of the cytoplasm, nucleus and nucleolus seen after the longer exposures is definite, but is found in relatively few cells. This cellular hypertrophy has been observed by Dominici (1909) (9) and Rubens-Duval (1914) (10) who worked with radium, and by Lacassagne and Monod (1922) (2) who worked with X-rays.

(4) The changes which take place in some cells, described as "breaking down cells," are of interest. These changes appear to take place in growing



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cells which have been damaged by exposure to the X-rays and to develop when the cell is about to divide, that is shortly before prophase, but in some cells the "breaking down" does not take place until telophase is well established when one or both of the resulting daughter cells may break up. If the cell is watched on a warm stage the movement and changes of outline of the cytoplasm, nucleus and nucleolus is definite. The cells become disorganised and show most irregular and constantly varying forms. Parts of the cytoplasm and nucleus break up and disintegrate and eventually the whole cell appears to go into solution in the surrounding medium. Schaudinn (1899) (11) exposed different species of the lower organisms to the X-rays and watched the results under the microscope during exposure. He noted that the organisms *Amoeba lucida* and *Oxyrrhis marina* became globular, and after a certain length of exposure underwent sudden and violent disintegration.

(5) Even with two hours' exposure an immediate lethal dose was not given to any of the cultures although a few cells were destroyed.

(6) The changes in the cells do not appear to differ from changes found in cells which are growing in an unfavourable or modified medium.

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*The Measurement of Percentage Hæmolysis.—I.*

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MOST of the observations made on the rate of action of hæmolytic substances on red cells express the time taken, under various conditions, for the production of complete hæmolysis. While such observations are comparatively easy, it is a much more difficult matter to find the percentage number of cells hæmolyzed from time to time from the commencement of the reaction until its completion. The methods in use fall into two classes. In one method, the reaction is stopped after it has proceeded a certain length, the stopping being carried out as a rule by cooling, which greatly reduces the rapidity of hæmolytic reactions: the intact cells are centrifuged off, and the quantity of hæmoglobin in the supernatant fluid determined by matching in a colorimeter against a standard. From this, the percentage number of cells hæmolyzed is calculated. In the second method, less used on account of its greater inaccuracy, the degree of hæmolysis at various periods of time during which the hæmolysis is proceeding, is determined by comparing the appearance of the tube in which the reaction is taking place with a series of standard tubes, in which various quantities of cells—from 10 per cent. to 100 per cent. of the total number used—have been hæmolyzed.

The inaccuracy of the second method is obvious; it is not possible to judge the percentage hæmolysis by direct comparison except in a very rough way. The first mentioned method has also disadvantages, being unsatisfactory in three respects particularly. Cooling the tube in which the hæmolysis is proceeding does not stop the reaction, but merely retards it; further, this low temperature must be maintained until the intact cells are separated from the hæmolyzing fluid by the centrifuge; this is difficult to do without a specially constructed instrument. During the time taken to centrifuge, hæmolysis proceeds at a slow rate; the result is, therefore, that the values for percentage hæmolysis obtained by this method are too high. In the second place, the method is not satisfactory since the centrifuging causes many of the cells, weakened by the hæmolytic agent, to hæmolyse; this fact also tends to render the results obtained for percentage hæmolysis too high. The third disadvantage is the most important. It is evident, from the nature of the method, that it is

applicable only to hæmolytic reactions which proceed slowly; the method is therefore of very limited application. The study of percentage hæmolysis at high temperatures, or by means of hæmolytic agents in high concentrations, is impossible, because of the speed of the reaction.

It is desirable to have a method whereby the percentage number of cells hæmolysed from minute to minute can be rapidly determined, without the necessity of stopping the reaction in progress. Such a method is described below.

### *Principle.*

Since a suspension of red cells is opaque, whereas hæmolysed cells give a clear solution, the former will cut off more light than the latter. The quantity of light passing through a fluid containing cells will therefore vary with the number of cells hæmolysed, increasing as hæmolysis proceeds. The quantity of light passing through the fluid may be measured by observing the speed of rotation of a radiometer, exposed to it, and sufficiently screened from heat.

### *Description of Apparatus.*

The apparatus used must meet certain requirements: (1) the source of light must be constant; (2) the cell containing the red cell suspension to be hæmolysed must be water-jacketed, so that hæmolysis may be carried out at any desired temperature; (3) the radiometer used must be sensitive to small changes in the intensity of the light; (4) the radiometer must be screened against the heat proceeding from the source of light. These requirements are met by the apparatus here described.

The source of light used is a gas-filled lamp, giving 1,000 candle-power. It is enclosed in a box, fitted with a large funnel above and ventilation holes below, so as to diminish as far as possible the heat. The lamp is controlled by a switch. It is mounted on a rod, terminating in a screw, so that it may be moved away from or towards the cell containing the erythrocytes to be hæmolysed.

The light from the lamp passes first through a large tank (A, fig. 1). 9 inches broad, 8 inches high, and 2 inches thick; the front and back walls of this tank are made of glass. The tank has an inlet and an outlet; these are connected to the cold water supply, so that a constant stream of water passes through, the object being to prevent heat from the light from reaching the water-jackets of the hæmolysis cell. After passing through the tank the light falls on an aperture,  $2\frac{1}{2}$  inches square, in the front wall of the box in which the lamp is enclosed, and situated in the direct line of the light. It then traverses

the water-jackets of the hæmolysis cell and the cell itself. The first water jacket of the hæmolysis cell is 3 inches long, and  $2\frac{1}{2}$  inches square on cross-

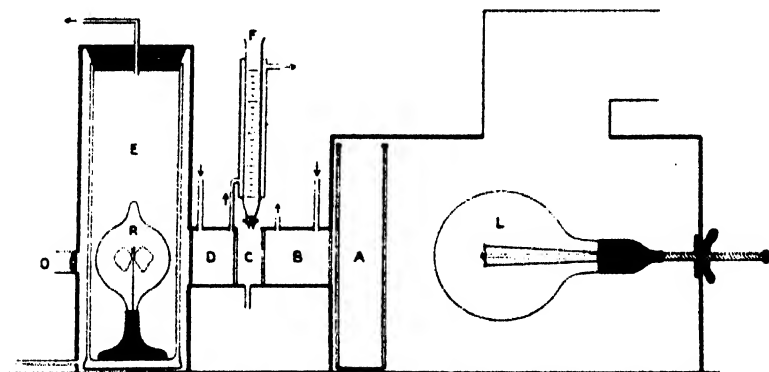


FIG. 1.

section. The walls which lie in the path of the light are of plate glass. This chamber has three holes in its roof, through one is passed a thermometer, one carries an inlet tube, while the other carries an outlet tube. These holes are situated, not in the middle line of the chamber, but near the side walls. The hæmolysis cell, lying between the chamber just mentioned and a second similar one, is  $2\frac{1}{2}$  inches square in cross section and 1 inch thick. Its capacity is almost exactly 100 c.c. The top of this chamber is open, the bottom is perforated with an outlet tube with a tap. On the side of the hæmolysis cell far from the light is a second chamber similar to the first, but measuring 2 inches in length instead of 3 inches. The walls in the path of the light are of glass; as in the case of the first chamber, it has an inlet, an outlet, and a hole for a thermometer in the roof. The two water-jacket chambers and the hæmolysis cell are all made in one piece, a metal trough being constructed with four glass plates dividing it into three chambers. The two end chambers have their tops covered in with celluloid plates perforated with the apertures necessary. These chambers are all made water-tight. In this way the light passing from the lamp towards the radiometer passes through the glass plates forming the walls of first the first water-jacket, then the cell, and finally the second water-jacket. In the diagram, the water-jacket chambers are B and D, the cell being marked C. The light having passed through these chambers, falls on a window  $2\frac{1}{2}$  inches square in the wall of the box containing the radiometer, passing through this it falls on that instrument in its water-jacket.

The radiometer used is a matter of great importance. Two points are essential: it must be sensitive to small differences in intensity of light, and its

vanes must be at right angles. The sensitiveness can only be determined by trial. Over forty radiometers were tried before the instrument used in connection with this investigation was selected. The sensitiveness to heat is very great. It therefore is necessary to enclose the instrument in a chamber containing running water, at a constant temperature. The glass vacuum chamber containing the vanes is fitted to a heavy brass base, which is attached to the bottom of a glass jar large enough to hold the instrument. The jar has an inlet below, and an outlet above, it being sealed at the top with a rubber stopper. The jar used is 3 inches in diameter and 10 inches high. Through this jar, when the radiometer is in use, a stream of cold water, at a constant temperature, is kept constantly running. Thermometers placed in the inlet and outlet tubes allow the temperature to be kept under observation. (In diagram, radiometer R in water-jacket E.)

The radiometer in its water-jacket is enclosed in a box large enough to hold it. In the side of the box near the lamp is the aperture described, through which the light falls on the vanes of the instrument. In the direct line of the light, and in the opposite wall to this aperture, is a small metal tube containing a lens through which the vanes of the radiometer may be seen, and also a piece of blue glass to diminish the intensity of the light. The lamp, the centre of the hæmolysis cell and its water-jackets, the vanes of the radiometer, and the lens are all in alignment.

All surfaces of the chambers through which the light passes—except, of course, the glass plates—as well as the interior of the radiometer box, are blackened with a dead black varnish. All the parts of the apparatus so far described are fastened rigidly together, so that their position with regard to each other shall not alter, for any alteration would affect the path of the light.

Above the hæmolysis cell, which has an open top, is arranged a short burette with a tap (F), surrounded by a water-jacket, which is filled with water passing from the outlet of chamber D, and which after flowing round the walls of the burette, escapes. In this burette, the cells to be hæmolysed are placed. By means of the water-jacket they are brought to the same temperature as that in chambers B and D, and therefore at any given moment the warmed cell suspension may be added to the hæmolytic agent in the hæmolysis cell, at the same temperature. The capacity of the burette is 25 c.c.; it is large in diameter, so as to reduce the length and avoid heat loss as far as possible. It is provided with a thermometer.

The two chambers, B and D, on either side of the hæmolysis cell C, have

their inlet pipes attached to the cold water supply ; the pipe made of thin glass which carries the water is wound spirally in a vessel (V in fig. 2), containing water, heated by a gas flame, which is controlled by an automatic regulator in the vessel. The regulator can be adjusted to any temperature required. By suitably regulating the flow of water and the temperature of the water in the vessel, any temperature desired, from about  $3^{\circ}\text{C}.$  to over  $40^{\circ}\text{C}.$ , can be produced in chambers B and D, and also in the water-jacket of the burette F. Fluid in the hæmolysis cell C quite rapidly acquires the temperature of the chambers on either side of it. The water, after passing through chamber D, passes into the water-jacket of the burette, and thence escapes, that passing through chamber B escapes by the outlet tube.

Into the hæmolysis cell, close to the side wall, passes a glass tube, drawn to a fine capillary. This tube carries compressed air, so that when fluid is in the cell, a stream of bubbles passes constantly up the side of the cell, and keeps the erythrocytes from settling. The hæmolysis cell is also provided with a thermometer, suspended near the side wall, so as to be out of the direct line of the light.

#### *Technique.*

Before carrying out an experiment, the apparatus must be prepared for use and calibrated.

*Preparation.*—The tank in the lamp box (A) is filled with water, the water being allowed to circulate through it until the calibration and the experiment are finished. The water supply to the radiometer water-jacket (E) is also turned on, the temperature registered by the thermometers at the inlet and outlet tubes being noted. The water is allowed to circulate through this water-jacket for the whole time of the calibration and the experiment, neither of which must be carried out till the temperature of the outflowing water is the same as that of the inflowing water, and until the temperature of both has ceased to fall. This usually takes about half an hour. The water, being supplied from the main cold water supply, is very constant in temperature. The chambers B and D are now filled with water, which is allowed to circulate slowly through them while the calibration and the experiment are being carried out. The chambers, if properly constructed, fill completely, so that there is no air space near the roof. The temperature of the water passed through them depends on the temperature at which it is desired to produce hæmolysis. The stream of water is warmed by heating the vessel V to the required temperature, and setting the automatic gas regulator. By adjusting the temperature of the vessel containing the spiral, and also by regulating the

stream of water, the temperature of the chambers on either side of the hæmolysis cell may be rendered constant in about fifteen minutes. The temperature of

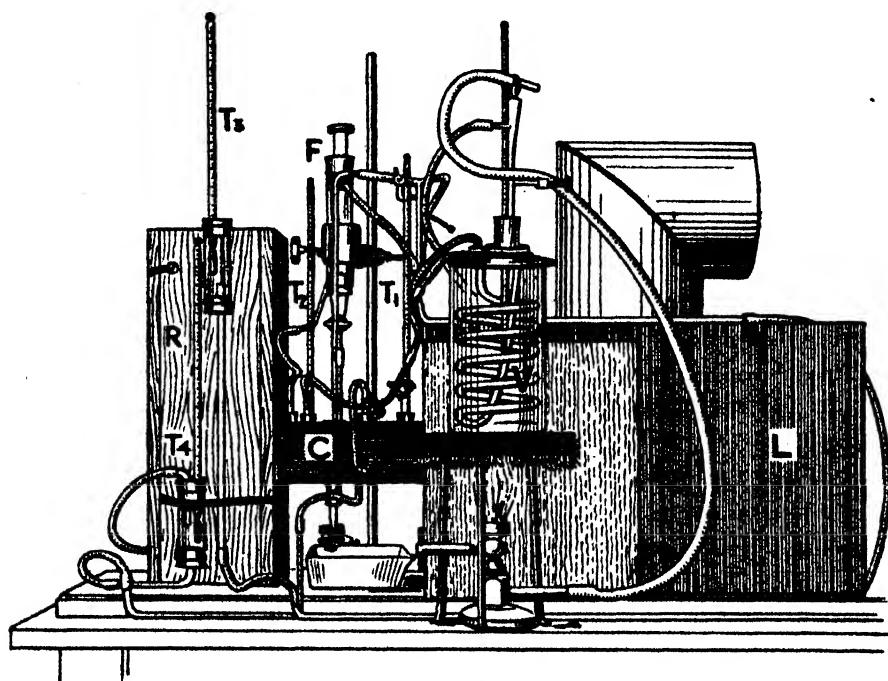


FIG. 2.

the chambers is read off on the thermometers with which each is supplied. When the temperature in the radiometer water-jacket is constant, and when that of the chambers B and D is what is needed for the experiment, the apparatus is ready for use.

*Calibration.*—This consists of finding the relation between the speed of rotation of the radiometer and the percentage number of erythrocytes hæmolysed in the fluid contained in the hæmolysis cell.

The concentration of red cells to be hæmolysed is obviously of great importance. If too many cells are present in the fluid, so much light will be cut off that the amount passing through will be insufficient to set the radiometer in steady motion. If, on the other hand, the concentration of erythrocytes is too small, there will be an insufficient difference between the speed of the radiometer when no cells are hæmolysed, and when complete hæmolysis is reached. In all other papers by the writer, a standard suspension of red cells has been used ; this consists of the cells contained in 1 c.c. of blood suspended—

after washing—in 20 c.c. of saline. For the purposes of the experiments on percentage hæmolysis dealt with in this paper, this suspension is too strong. One which contains a quarter of the number of erythrocytes gives excellent results.

A series of standards are made as follows, in a number of bottles of capacity of about 150 c.c. In each of ten bottles is placed 50 c.c. of distilled water. To the first bottle is added 0.025 c.c. of blood from the finger, to the second 0.05 c.c. of blood, to the third 0.075 c.c. and so on, the amount of blood added to each bottle being 0.025 c.c. more than to the previous one, so that the tenth bottle has added 0.25 c.c. of blood. The blood must flow freely from the finger. The bottles are allowed to stand for a few minutes; the red cells of the blood added are, of course, hæmolyzed. To each bottle is now added 50 c.c. of 1.7 per cent. NaCl in distilled water; this makes the contents of each bottle isotonic. Blood is added to each bottle from the finger, so that the quantity of blood added to each is altogether 0.25 c.c., that is, to the first bottle will be added 0.225 c.c. of blood, this amount decreasing by 0.025 throughout the series, so that no blood is added to the tenth bottle. These bottles now contain solutions, in which are both hæmolyzed cells and intact cells, so as to constitute a series of standards, representing the conditions of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 per cent. hæmolysis, which would exist if 20 c.c. of a red cell suspension containing a quarter of the number of the red cells in the standard suspension usually used in the writer's researches, were added to 80 c.c. of saline containing a hæmolytic agent.

One other standard is prepared, to represent 0 per cent. hæmolysis, by adding 0.25 c.c. of blood to 100 c.c. of saline.

The standards being prepared, the light is switched on, and the calibration is carried out as follows. The fluid representing 0 per cent. hæmolysis is placed in the hæmolysis cell, which it should fill to the top, so that no light passes above the level of the fluid. The compressed air is turned on, so that a gentle stream of bubbles pass through the fluid. After about a minute, the time taken for the rotation of the radiometer is taken with a stop watch. Two or three observations should be taken, at intervals of about three minutes, in order to ensure that the rate observed is constant. The readings should not differ by more than three seconds for this standard, and as a rule are closer than this. The fluid is then drained out of the hæmolysis cell, and replaced by the next standard solution. The speed of the radiometer corresponding to this standard is observed in the same way; it will be greater than in the case of the first solution. This process is repeated, until the rate of revolution

of the radiometer corresponding to each of the eleven standards is obtained. The average of three readings is taken. These readings should not differ by more than two or three seconds in the case of standards representing 0 to 40 per cent. hæmolysis; from 50 to 70 per cent. hæmolysis, by not more than one second, while for the standards in which hæmolysis is more complete the speed of revolution should be constant to a fifth of a second. It is not difficult to obtain this degree of accuracy. Great care must be taken that the temperature in the radiometer water-jacket is constant, that the compressed air keeps the red cells in circulation, and that the hæmolysis cell is full, so that no light passes over the fluid.

Using the suspension of cells mentioned above, the speed of the radiometer when hæmolysis is complete is four times greater than that when there is no hæmolysis, as can be seen by reference to the calibration of the apparatus in connection with some of the experiments quoted below.

From the observed times taken for the completion of one revolution of the radiometer vanes, a graph is constructed showing the relation of the speed of rotation to the percentage hæmolysis, when the water-jacket of the radiometer is at a certain temperature. This completes the calibration, which is better carried out before each set of experiments, but which may be done less frequently. It does not take long to do, and ensures that the apparatus is in good order; further, the fact that frequent calibration curves coincide closely gives a confidence in the results obtained for the experiments.

*Technique of experiment.*—The apparatus being prepared, the chambers on either side of the hæmolysis cell constant at the temperature at which it is desired to carry out the experiment, and the calibration being completed, the curve for percentage hæmolysis for a given dilution of hæmolytic agent acting on the red cell suspension may be obtained.

The hæmolytic agent to be employed is dissolved in 0.85 per cent. NaCl, so that the concentration is five-fourths of that required; 80 c.c. is placed in the hæmolysis cell. At the same time 20 c.c. of the blood suspension to be used is placed in the burette above this cell. This suspension is one-fourth the concentration of that usually used, and is prepared by receiving 1 c.c. of blood into citrated saline, centrifuging off the cells, washing these three times with 0.85 per cent. NaCl, and suspending the washed cells in 80 c.c. of saline. After allowing the hæmolytic agent and the blood suspension to come to the desired temperature, as indicated by the thermometers dipping into each, the cells are added to the hæmolyzing fluid; the time is noted. The compressed air should be turned on, so as to keep the cells mixed evenly in the fluid. The



light is now turned on. After a minute has elapsed, the speed of the radiometer is taken with a stop-watch. From minute to minute the speed is determined; as hæmolysis proceeds, the rate of the revolution of the vanes becomes faster, until complete hæmolysis is reached. From the data obtained a curve is constructed showing the speed of rotation of the radiometer from minute to minute, from the beginning of the reaction until its completion. The temperatures registered by the various thermometers are noted as the reaction proceeds. No change should occur.

If the reaction under observation is slow—for example, if complete hæmolysis is not reached until twenty or more minutes have elapsed—it is sufficient to determine the rate of rotation of the radiometer, not each minute, but every three minutes. If the reaction be very slow, it is sufficient to take readings at even longer intervals. If the reaction be rapid, a greater number of readings may be taken by observing, not the time taken for a complete rotation of the radiometer, but that required for a half rotation; it is better, however, to take the complete revolution if possible.

Two curves being now obtained, one showing the relation of the speed of rotation of the radiometer to percentage hæmolysis, and the other showing the rate of rotation of the radiometer from minute to minute from the beginning of the reaction until its completion, it is obviously a simple matter to find the percentage hæmolysis produced by the hæmolytic agent from minute to minute. The graphical expression of this gives what may be termed the curve of percentage hæmolysis for the particular dilution of hæmolytic agent acting on the quantity of cell suspension used, at the temperature at which the experiment is conducted.

After the completion of the experiments, all the chambers of the apparatus which contain fluid should be emptied, since, if water be allowed to stagnate in them, moulds make their appearance on the glass walls of the chambers, the cleaning of which is difficult.

The preparation of the apparatus, the calibration, and the obtaining of the percentage curve for the hæmolysis, are all quite easily carried out. After a little experience, the method is easier than that whereby the intact cells are centrifuged off, and if a number of curves are to be obtained, much more rapid. It will, of course, be obvious that a much greater number of points on a curve can be determined by this method than by any hitherto used. Further, all the disadvantages of these older methods are eliminated. The percentage error attached to the determinations of percentage hæmolysis by the radiometer vary according to the degree of hæmolysis. From 0 to 25 per cent.

hæmolysis the results are not so reliable as for degrees of hæmolysis greater than this, there being about 3 per cent. error in this range. Thereafter the error decreases with advancing hæmolysis, so that when about half the cells are hæmolysed, the readings are correct to from 1 to 3 per cent.; when hæmolysis is nearly complete the greatest error is 1 per cent. It is of importance that the point of least accuracy is in the region of 20 per cent. hæmolysis, for though a small error here does not affect the curve to any appreciable extent, it is sufficient to render certain methods of expressing the results valueless. This point will be referred to later.

*Results obtained.*

It is not intended in this paper to give an exhaustive account of the curves obtained in connection with various hæmolytic agents acting under various conditions, but rather to describe certain important and typical results, to discuss their significance, and to show their connection with the results obtained by other observers.

It is first of all desirable to ascertain to what degree the results obtained by the method described above and by the method of centrifuging off the intact cells, are in agreement. This is shown in the following experiment.

*Experiment 1.*—Hæmolysis of red cell suspension, at 11° C., by 1 in 40,000 saponin.

Time in minutes.	Percentage Hæmolysis.	
	Radiometer method.	Centrifuge method.
0	0	0
2	0	—
5	14	16
6.5	22	—
9	43	—
10	55	60
12	76	—
13	83	85
15	92	—
18	98	98
20	100	100

The correspondence is very close, considering the difficulties attached to the centrifuge method. The points obtained by that method are a little more irregular than those obtained by the radiometer method, besides necessarily being fewer; they fall, however, very nearly on the same curve. This close

correspondence has been found on many occasions. It is usual for the centrifuge method to give results a little higher than those given by the radiometer, especially for low values of hæmolysis. The differences are, however, never great enough to affect the general form of the percentage hæmolysis curve.

The results obtained with hæmolytic agents may now be considered. Experiments will be given in full showing the relation between percentage hæmolysis and time in the case of three hæmolytic substances: sodium hydrate, saponin, and sodium taurocholate. These substances have been chosen because the curves obtained by investigating their action illustrate certain important points to be dealt with in the discussion. The dilutions in which the substances were allowed to act on the red cells were adjusted so that the time for complete hæmolysis should be nearly the same in each case, with the purpose of rendering the results readily comparable.

*Experiment 2.*—Hæmolysis of red cells by N/200 NaOH at 12.5° C.

(a) *Calibration curve*:—(97,0), (77,10), (65,20), (54,30), (47,40), (41,50), (36,60), (32,70), (28,80), (26,90), (25,100).

The ordinate in all the calibration curves represents percentage hæmolysis in the standard, the abscissa representing the number of seconds required for one complete rotation of the radiometer.

(b) *Experimental curve*:—(97,0), (97,5), (81,10), (47,15), (30,18), (26.5,20), (25.5,22), (25,27).

On the ordinate is plotted time in minutes from the beginning of the reaction; on the abscissa is plotted seconds required for one revolution of the radiometer.

(c) *Curve for percentage hæmolysis*:—(0,0), (5,0), (10,8), (15,40), (18,75), (20,88), (22,94), (27,100).

On the ordinate is plotted percentage hæmolysis; on the abscissa, minutes from the beginning of the reaction. This curve is derived from (a) and (b) by an obvious process. The curve is shown in fig. 3, p. 396.

*Experiment 3.*—Hæmolysis of red cells by 1 in 50,000 saponin at 14° C.

(a) *Calibration curve*:—Same as in Experiment 2.

(b) *Experimental curve*:—(98,0), (97,2), (94,3), (85,6), (57,9), (40,12), (30,15), (26.5,18), (25.2,98), (25,27).

(c) *Curve for percentage hæmolysis*:—(2,0), (3,1), (6,5), (9,25), (12,52), (15,75), (18,88), (24,98), (27,100).

This curve is shown in fig. 5, p. 398.

*Experiment 4.*—Hæmolysis of red cells by 1 in 7,000 sodium taurocholate at 14° C.

- (a) *Calibration curve* :—(90,0), (76,10), (64,20), (54,30), (46,40), (41,50), (38,60), (32,70), (28,80), (25,90), (100,21).
- (b) *Experimental curve* :—(90,0), (88,5), (83,7), (60,13), (49,15), (40,16), (34,17), (29,18), (26,19), (24,20), (22,21), (21,22).
- (c) *Curve for percentage hæmolysis* :—(0,0), (4,0), (5,1), (7,5), (13,24), (15,35), (16,52), (17,65), (18,78), (19,86), (20,92), (21,98), (22,100).

This curve is shown in fig. 7, p. 401.

### Discussion.

Before discussing the results obtained, it will be convenient to consider the investigations of other workers. Certain of the results obtained in connection with bacteriolysis will be considered along with those dealing with percentage hæmolysis.

Many observers have stated that the formula describing monomolecular reactions,  $n_t = n_0 e^{-kt}$  applies to hæmolysis and to the lysis of bacteria.  $n_0$  being the number of cells or bacteria at the beginning of the experiment, and  $n_t$  the number surviving after time  $t$ ;  $k$  is a constant. Arrhenius (1) states that this formula applies equally to bacteria and to red cells. He explains that it might be expected that bacteria would have varying resistances to the action of a lysin, some being very resistant, others very weak, while the majority possessed an intermediate resistance. He also points out that if this were the case, instead of the straight line which results if the logarithm of the number of surviving bacteria after various times be plotted against the time in cases where the monomolecular formula applies, a curved line with a double inflection would result. This latter curve Arrhenius demonstrates not to be obtained in the case of bacteriolysis. It is therefore to be concluded that the natural resistances of the cells is a matter which does not affect the reaction in any way. That the monomolecular formula applies also to hæmolysis is demonstrated; it is, however, shown that the red cells examined were of different natural resistances, the resistances being distributed according to the law of probability. How this statement is to be reconciled with the fact that the plotting of the logarithm of the number of surviving cells against the time results in a straight line, is not explained. In fact, a contradiction is involved. The experiments by which it was shown that the monomolecular formula is applicable are very unsatisfactory, since the reaction was frequently not

observed throughout its whole course, but only for a limited time. This is a frequent fallacy in Arrhenius' investigations on hæmolysis. Henri (2) finds that the monomolecular formula applies to hæmolysis; he neglects the influence of individual variation as regards resistance, for which he has been severely criticised by Mioni (3), whose experimental evidence is, however, equally inconclusive. Dreyer and his co-workers (4, 5) observe that, after a period which they term the period of induction period, the monomolecular law is followed. This period of induction is to be carefully distinguished from the period of incubation referred to by Arrhenius. During the former, hæmolysis proceeds, but not according to the monomolecular law. During the latter, no hæmolysis occurs at all. The period of induction cannot be regarded as anything but a portion of the curve describing the reaction, to which it is not possible to fit a convenient and simple formula. Several explanations have been advanced to explain the supposed fact that the lysis of red cells, which are of varying resistances, is described by a typical monomolecular curve. The most interesting of these is that of von Liebermann and von Fenyvessy, whose explanation is that the exit of hæmoglobin from the red cell is governed by the relative concentrations of that substance inside the cell and outside it respectively. This explanation neither meets the case nor is supported by facts.

Allied to the problem of percentage hæmolysis is that of percentage bacteriolysis. Madsen and Nyman (6), Chick (7), Phelps (8), and Eijkman (9) state that this process is described by the formula for monomolecular reactions. Most of these observers recognise that the typical monomolecular formula ought not to apply, since the bacteria have different resistances to the destructive agent. Madsen and Nyman admit the fact and then ignore it. Chick imagines the cells to undergo rapid cyclic changes in their resistance, an explanation which Eijkman and Phelps adopt. The explanation not only has no evidence to support it, but is so unsatisfactory that Chick herself modifies it in a later paper (10). The modification is not an improvement; indeed, if true, it proves the opposite of what Chick assumes, which is that the bacteria are all of one resistance. Robertson has also advanced a hypothesis (11), involving an impossible assumption, that "the number of units of underlying change," which he terms the quantity  $X$ , must be an exponential function of the time and also, at the same time, a constant.

Brooks (12) investigated the action of ultra-violet radiations on the red cells, and also the action of a hæmolytic serum. He found that the curves describing the reaction were sigmoid, and concluded that the course of the

process was largely dependent on variation of resistance to the hæmolysing agent among different cells. He also concluded that there is no evidence for the statement that such processes occur according to the monomolecular law, with which they cannot be reconciled except by strained hypothesis. Brooks' paper contains a very valuable discussion of the evidence, and a clear presentation of the mathematical theory. Brinkman (13) finds that the type of curve obtained in experiments dealing with the resistance of red cells to osmotic influences shows that some of the cells possess a different resistance to others, the majority being of a resistance between two extremes. Pasteur, Vallery, Radot and Lheriter (14) have shown that the resistance of red cells varies directly as their size; that the resistances will be distributed according to the law of probability will follow from this statement, if it be true; for the size of red cells is distributed in this manner, so far as can be judged, from the not very extensive investigations on this point—for example, the figures of Malassez. Lastly, it may be again emphasised that Arrhenius demonstrated the resistance of red cells to be distributed according to the law of probability.

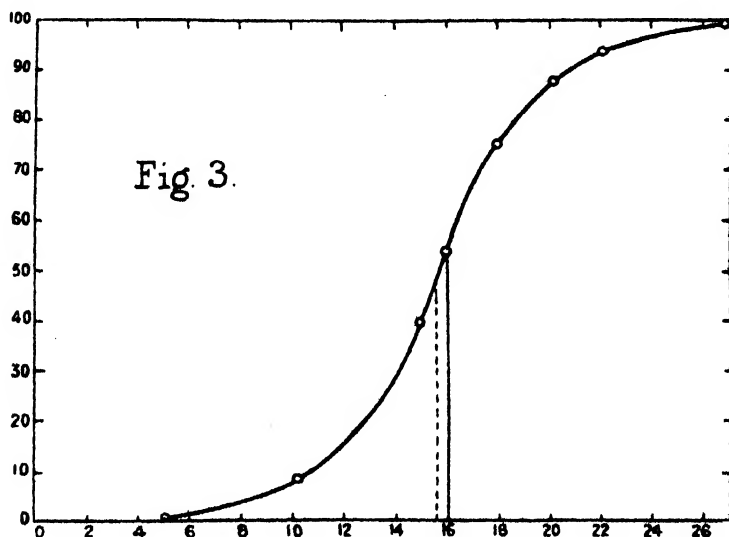
The recent work of Peters (15) on the death-rate of protozoa when in contact with  $\text{HgCl}_2$ , may also be noted in this connection. Peters stresses the fact that the organisms have resistances distributed probably according to a skew frequency curve. The results were obtained by counting the organisms, so that the figures are based on rather small numbers. There is, however, no doubt about the type of curve, or about the fact that its explanation lies in the difference of resistance of the groups of organisms. Peters notes that a considerable part of the reaction may be described by the monomolecular formula. The part which cannot be is equivalent to Dreyer's induction period; a purely artificial division of a curve in this way can have no advantage.

It is remarkable that only one of these observers considers the possibility that the hæmolysis may result in the production or liberation of a substance which may influence the velocity of the reaction. Brooks observes that the asymmetry of the sigmoid curves obtained may be due to an inactivation of the hæmolytic substance, this causing a retardation of the reaction.

Summarising the results discussed, one group of observers considers that hæmolysis and bacteriolysis are describable by the monomolecular formula, and that the curves obtained when percentage hæmolysis is considered are such as would be expected if a reaction of this sort were in progress. A second group considers that sigmoid curves are typical, and explain these as the result of a distribution of the resistances of the cells according to one form or other of a frequency curve. The only satisfactory attempt to discover the type of

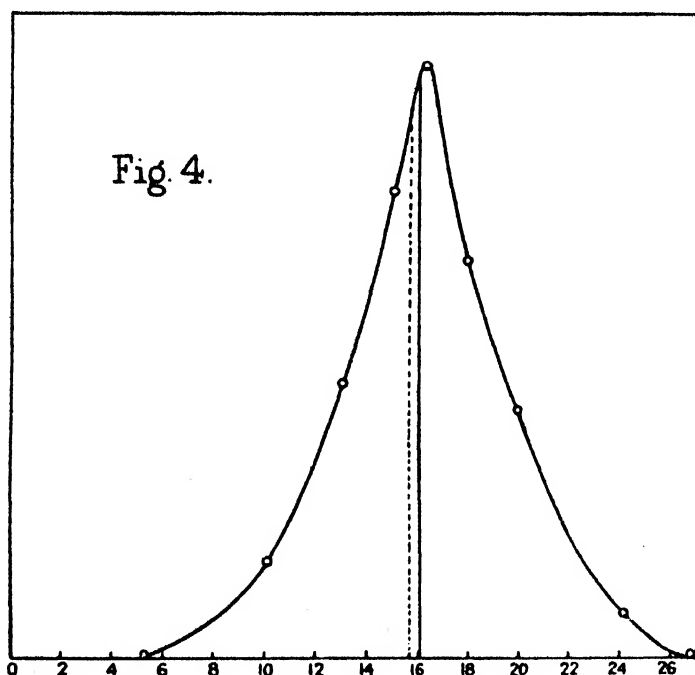
reaction which, taken together with the distribution of the resistances, would result in such sigmoid curves, is that of Brooks who, however, rightly limits his discussion to theory, since, as he states, inhibitory factors or other considerations may influence the experimental curves.

Considering now the curves obtained as the result of experiments recorded in this paper, it will be convenient to deal first with the observations on the percentage hæmolysis curve obtained when sodium hydroxide is used as a hæmolytic agent, since this is the simplest case. The experimental curve is shown in fig. 3. There is a distinct latent period, during which no hæmolysis



occurs. This is commonly found in experiments with hæmolytic agents which act slowly. After this latent period, the reaction resulting in hæmolysis commences, and is described by a sigmoid curve, almost symmetrical. The deviation from symmetry is so small as to fall within the range of the experimental error. The median of the curve nearly coincides with the ordinate erected at a point half way between the beginning of the hæmolysis and its completion. The median is indicated by a dotted line, the ordinate referred to by a black line. If the position of the quartiles be examined they will be found to be very nearly equidistant from the median. From this curve, the curve of the first derived function was obtained by graphical differentiation. It is shown in fig. 4, and is obviously very like a curve of a symmetrical frequency distribution; the resistances of the cells, measured by the time taken to hæmolysise them, being ideally distributed. As follows from the remarks made about the

integral curve from which this curve is derived, the median lies very close to the ordinate erected at the point of time half way between the commencement



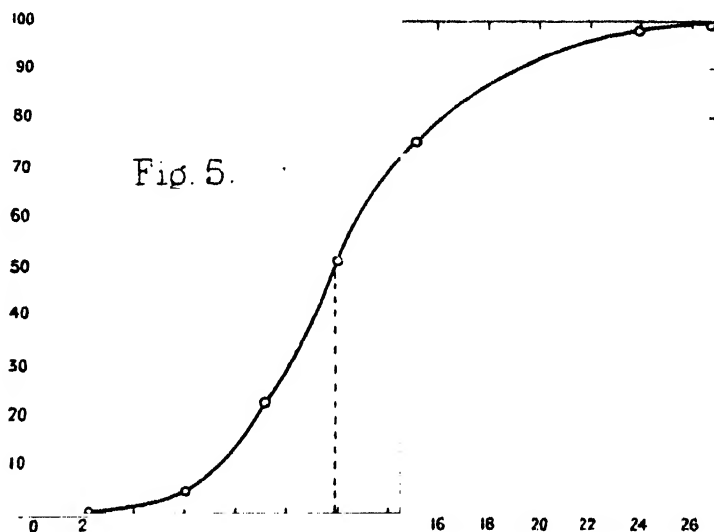
of the hæmolysis and its completion, while the quartiles are equidistant from the median (indicated by dotted line). The deviation of both from the highest ordinate of the curve is so small as to be accounted for by a small and allowable experimental error, or by the error involved even in careful graphical differentiation.

The chief difficulty underlying the attempt to ascertain the nature of the hæmolytic reaction lies in the fact that, in the case of the hæmolytic agents usually employed for experiments of this kind, the curve relating to percentage hæmolysis to time is not symmetrical. This fact may be explained according to one of two hypotheses: that the reaction proceeds with a constant velocity, and that the distribution of the red cells is expressed by a frequency curve exhibiting skewness—it may be noted that the frequency curve obtained by Arrhenius for resistance to hæmolysis by vibrolysin exhibits scarcely any skewness—or, that the reaction is monomolecular, and that the resistances are ideally distributed. Now in the case under consideration there is no stry. It is accordingly correct to conclude that the hæmolysis produced



by sodium hydroxide proceeds with a constant velocity, and that the distribution of the cell resistances is according to a symmetrical frequency curve. It is impossible to assume that the reaction is monomolecular.

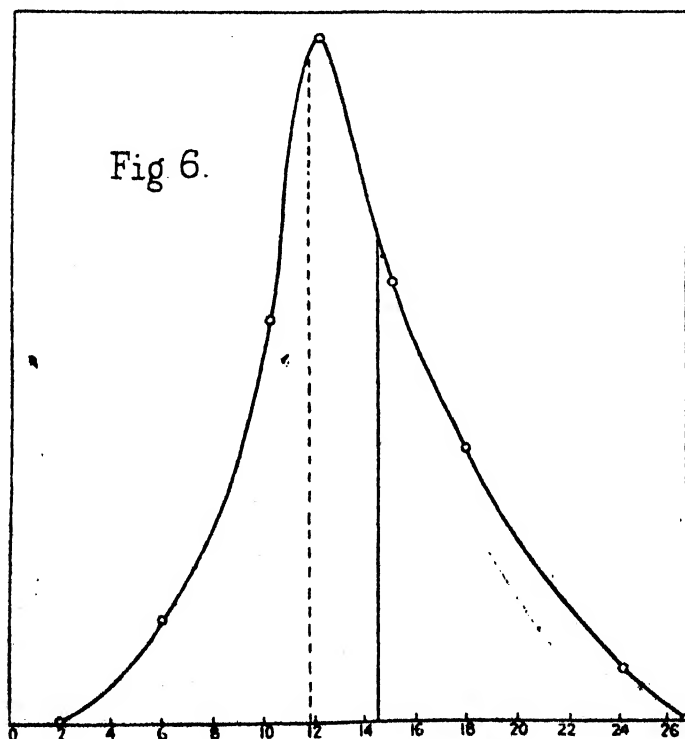
Proceeding now to the curve relating percentage hæmolysis to time when saponin is used as a hæmolysing agent, and shown in fig. 5, it will be observed



that the curve is of the type usually obtained with hæmolytic substances such as sera and ultra-violet radiations. There is a considerable amount of asymmetry, the number of red cells destroyed in the first half of the reaction being greater than that hæmolysed during the second half. This type of curve might be explained by assuming a monomolecular reaction to be taking place, the red cell resistances being considered ideally distributed. There is no necessity to introduce the idea of a monomolecular reaction in the case of saponin, when such an explanation is not applicable to hæmolysis produced by other hæmolytic agents, such as the alkalis, for there is direct evidence that the process of hæmolysis results in liberation of substances from the cell which have a retarding effect on the fundamental reaction. This fact supplies a very much simpler explanation of the type of curve produced. The results may be explained by considering the fundamental reaction the same as that which occurs when cells are hæmolysed by alkali—of constant velocity—the resistances of the cells being, as in the cases of alkali hæmolysis and hæmolysis by vibriolysin, ideally distributed, and allowing for the fact that the fundamental reaction becomes retarded by

the fact that hæmoglobin, and also, possibly, other proteins contained in the red cell, is liberated in increasing amounts as hæmolysis proceeds, it having been shown that the proteins of serum and also hæmoglobin render saponin non-hæmolytic (16), so that the quantity of hæmolytic substance acting on the cells towards the end of the experiment will be much less than that acting at the commencement. A retardation in the reaction must therefore occur. This fact is quite sufficient to account for the shape of the curve without postulating a monomolecular reaction, or a skew distribution in resistances, for neither of which assumptions is there any evidence, and both of which involve difficulties when applied to hæmolysis in general.

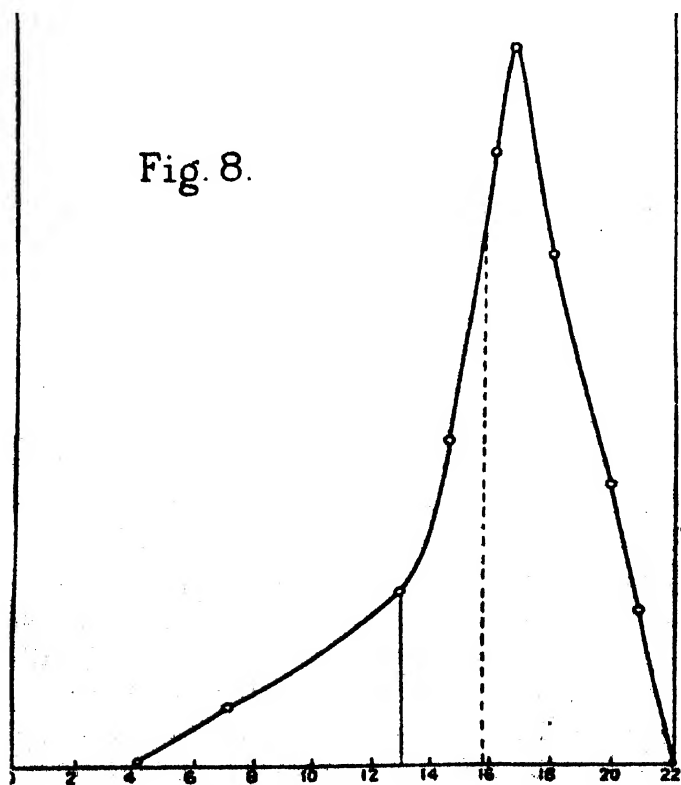
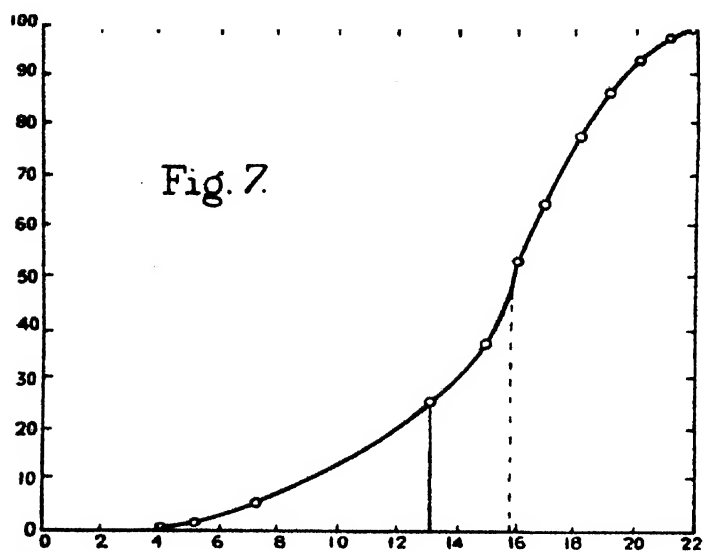
In fig. 6 is shown the curve of the first derived function of the curve in fig. 5,



obtained by graphical differentiation. It has the appearance of a slightly skew frequency curve. The median, indicated by a dotted line, is considerably removed from the line, indicated in black, showing the mid point of the time axis over which the reaction proceeds. The median almost coincides with the maximum ordinate. All these points suggest that the asymmetry of the curve is due to a retardation in the velocity of the reaction as it proceeds, the retarda-

tion becoming marked only after hæmolysis of the bulk of the cells, as would be expected if the slowing of the reaction were due to the retarding action of substances liberated from the cells by the hæmolysis. The skewness of this curve is  $-0.16$ . A simple method was used in order to find the degree of skewness—that given by Bowley (17). It is true that this method is not as sensitive as the method more commonly used, but it is sufficiently accurate when the nature of the data is considered. There is one disadvantage connected with it. Since it depends on the distance of the quartiles from the median, a small experimental error in the region of the quartiles conveys a considerable skewness. As has been pointed out, the curves obtained by the radiometer are least accurate in the region of the lower quartile; measurements of skewness are thus liable to be somewhat inaccurate; the differences in the curves dealt with in this paper are, however, so great as to allow this possible error to be ignored. It will be noticed that there seems to be a distinct latent period before hæmolysis commences. This, however, is shorter in the case of saponin than in the case of most hæmolytic agents. Brooks objects to the postulation of latent and induction periods in connection with these curves, on the grounds that there is no proof for their existence. This is certainly true of induction periods, which appear to be merely artificial subdivisions of the curves in which they are supposed to exist. There seems to be, however, quite a definite period before any hæmolysis is detectable, and it seems reasonable, when considering the percentage hæmolysis in relation to time, to consider this period as being distinct from the curve representing the relation.

The results obtained when sodium taurocholate is used as a hæmolysing agent may now be considered. Here the curve representing the relation between percentage hæmolysis and time, shown in fig. 7, is of a different type to that obtained with sodium hydroxide, or to that obtained with saponin. It is unsymmetrical, but in the opposite way to the saponin curve; this is clearly seen in fig. 8 where the graph of the first derived function is shown. The number of cells hæmolysed during the first part of the time during which the experiment proceeds is less than the number hæmolysed during the later stages. It is impossible to consider this type of curve as resulting from a monomolecular reaction. It might be produced by the taurocholate acting with a constant velocity on cells whose resistances were distributed according to a skew frequency curve, but the degree of skewness necessary would be about  $+0.45$ , a degree which is so improbable that very strong evidence would have to be led before such an explanation could be even considered. An alternative explanation is to suppose the reaction to be one which, if uninterfered with, would



proceed at a constant velocity, and the distribution to be ideal—both the distribution and the reaction being thus considered the same as has been found to meet the case in the curves for hæmolysis by sodium hydrate and by saponin—and to attribute the increase in the number of cells hæmolysed towards the end of the reaction to an acceleration of the action of the bile salt, produced by the same substances which account for the retardation in the case of saponin hæmolysis, the substances liberated from the cells as the result of hæmolysis. In this way, strained hypotheses regarding the nature of the fundamental reaction and the frequency distribution would be avoided.

It is, however, generally recognised that the action of serum proteins is to retard taurocholate hæmolysis, as they do saponin hæmolysis. It has further been shown that this effect is produced not only by the serum proteins, but also by hæmoglobin (16). Indeed that the inhibitory effect of hæmoglobin on taurocholate hæmolysis is much greater than its effect on saponin hæmolysis. It might therefore be considered absurd to explain an acceleration, such as appears in the curves for percentage hæmolysis when taurocholate is used, by suggesting that the very substance which is known to be inhibitory produces an acceleration. Attention must be drawn to a very important point. The demonstration that hæmoglobin or that the serum proteins have an inhibitory action on the action of taurocholate, has always been to add to the solution of the hæmolytic agent a quantity of the serum or the hæmoglobin, and then to determine the delay produced in the hæmolysis of added red cells. If the occurrences which take place when cells are being hæmolysed be considered, it will be seen that there is an important difference between them and the conditions of the experiment which demonstrates the inhibition. In the case of the latter experiment, hæmolytic agent has added to it serum or hæmoglobin, the cells being then added. In the case of cells undergoing hæmolysis, the hæmolytic agent is first in contact with the cells, the hæmoglobin being added, as hæmolysis proceeds, as the result of destruction of the cells. Such a difference as this cannot be ignored ; for it has been shown in a previous paper by the writer that the order in which certain substances are added in hæmolytic experiments involving inhibition and acceleration phenomena, is of great importance, determining whether acceleration or inhibition results. A substance, such as histamine, which greatly accelerates the action of sodium glycocholate if the latter be in contact with red cells, will act as an inhibitory substance if added to the cells before these are brought into contact with the bile salt (17). It is therefore possible that hæmoglobin and serum act similarly, as retarding substances if first mixed with the bile salt, and as

accelerating substances if added to the mixture of bile salt and cells. Such a hypothesis would explain the percentage hæmolysis curve for sodium taurocholate in a simple way. Fortunately the correctness of this hypothesis is also demonstrable in a simple way, as the following experiment shows.

*Experiment 4.*—The method used was similar to that employed in previous papers. The red cell suspension used was of the same strength as that employed for the experiments with the radiometer—a quarter the strength of the usual standard. In the cases where serum or hæmoglobin were added after the reaction had commenced, the addition was made after 30 seconds. The hæmoglobin solution was prepared from washed red cells, so that serum proteins should be removed; to remove any envelopes of red cells it was passed through a Berkefeld filter, and to remove fats, was repeatedly extracted with ether. The results are expressed in the following table:—

1 in 3,000 taurocholate	3.5 mins.
1 in 3,000 taurocholate, plus 0.025 c.c. serum, added before addition of red cells	32.0 mins.
1 in 3,000 taurocholate, plus 0.025 c.c. Hb solution, added before addition of red cells	12.0 mins.
1 in 3,000 taurocholate, plus 0.025 c.c. serum, added after addition of cells	0.6 mins.
1 in 3,000 taurocholate, plus 0.025 c.c. Hb. solution, added after addition of red cells	1.2 mins.

The temperature at which this experiment was carried out was 25° C. The quantity of hæmoglobin added was just that amount which would be liberated by 0.5 c.c. of the standard red cell suspension if hæmolysed. The results show plainly (a) that the effect of the addition of serum or of hæmoglobin to the taurocholate before the latter has come in contact with the cells is to retard the reaction, and (b) that the effect of either serum or hæmoglobin if added to the mixture of red cells and taurocholate is to accelerate the hæmolysis to a great extent. There seems to be a parallel between the phenomena observed with histamine and histidine, and also with serum albumin solutions, and these effects observed with serum and hæmoglobin, in that all these substances, if added to bile salts, retard the hæmolysis which the salt produces, whereas, if they be added to the salt after it has been in contact with red cells, a great acceleration of hæmolysis is produced.

It remains now to be shown that this peculiar phenomenon is not observed with saponin, but that serum and hæmoglobin act as inhibitory substances whether added before or after the saponin has come into contact with the

cells. This is shown in the following experiment, conducted in a manner similar to that adopted in Experiment 4.

*Experiment 5.*—The same serum, and the same solution of hæmoglobin, were used as in Experiment 4. The temperature at which the experiment was conducted was 25° C.

1 in 30,000 saponin	3.25 mins.
1 in 30,000 saponin, plus 0.025 c.c. serum, added before the addition of cells	37.0 mins.
1 in 30,000 saponin, plus 0.025 c.c. Hb solution, added before addition of cells	3.7 mins.
1 in 30,000 saponin, plus 0.025 c.c. serum, added after addition of red cells	37.0 mins.
1 in 30,000 saponin, plus 0.025 c.c. Hb solution, added after addition of cells	3.7 mins.

These results show that (a) serum and hæmoglobin act as inhibitory agents to saponin hæmolysis whether added before or after the saponin has come in contact with the red cells; (b) that the inhibitory effect of hæmoglobin is very slight. This agrees with the findings in a previous paper (16).

These two experiments cannot be considered as anything but illustrative experiments. A complete study of these acceleration phenomena is being made. The same type of occurrence is met with, although the degree varies, throughout wide ranges of concentration of both the hæmolytic agent and the serum or hæmoglobin solution. It is a simple matter to demonstrate the fundamental point.

It appears then, that the acceleration of the reaction which occurs with increasing hæmolysis when sodium taurocholate is used as a hæmolytic agent may be explained by considering the effect of the liberated hæmoglobin, an explanation which also is applicable to the curve obtained when saponin is used. It may be suggested that the form of the curve obtained with vibrolysin—a slightly asymmetrical sigmoid curve—is due to a reaction proceeding with a constant velocity, acting on cells whose resistances are ideally distributed, together with a slight inhibitory effect producing the asymmetry. The action of the serum proteins and of hæmoglobin on vibrolysin is not known, but the serum proteins have a slight inhibitory effect on the action of the allied substances, staphalolysin and tetanolysin (18). It is also possible that the form of the curve obtained when the action of an immune serum plus complement is considered (12)—a curve of considerable asymmetry, suggesting a retardation of the reaction—may be due to the action of inhibitory substances, for such antilytic bodies are known to be given off by cells (19);

this explanation can be only tentative, as the phenomena observed with immune serum hæmolysis are so complicated. The curves obtained by Brooks (12), when ultra-violet rays were used to produce hæmolysis, are of a similar type, but here a similar explanation probably does not apply, since irradiation of red cells seems to be followed by osmotic changes (20).

In all the cases in which there is accurate experimental evidence, it appears that the curves obtained for the relation of percentage hæmolysis to time may be explained by (a) considering that the reaction which results in hæmolysis has a constant velocity from the time when hæmolysis begins to the moment of complete hæmolysis, (b) that the resistances of the red cells to the action of the hæmolyisin, when measured in terms of time, are distributed very nearly according to an ideal frequency curve, and (c) taking into account the effect of substances liberated from the cells as hæmolysis proceeds.

It is certainly curious that the velocity should be constant. Three facts must certainly be taken into account, however, when an attempt is made to ascertain the nature of the fundamental reaction. (1) All the evidence points to the hæmolysis of the cells being due to an alteration in the protein component of the cell wall, in the cases of saponin and of taurocholate at least. The compound, however, does not appear to be a molecular one, but rather an adsorption compound (21,16). (2) It is a fallacy to assume that the completion of the fundamental reaction occurs at the same time as the completion of hæmolysis; this is certainly not the case. The fundamental reaction continues long after the complete lysis of all the cells. This point will be dealt with in a subsequent communication. (3) Certain observations, if correct, show that the hæmolytic agents tend to be greatly concentrated in the neighbourhood of the cells. Considering these facts, it would be surprising if a monomolecular formula described the course of percentage hæmolysis, or the relation between time required for complete hæmolysis and the dilution of the hæmolytic agent.

The deduction to be made from the form of the curve obtained when sodium hydrate is used as a hæmolytic agent—if the curve be considered as symmetrical, the distribution of resistance ideal, and the reaction of a constant velocity—is that hæmoglobin has no inhibitory effect on the hæmolysis, if added after the hæmolytic agent has been in contact with the cells. In point of fact, hæmoglobin exercises a very slight inhibitory effect, very small compared to that which it has on saponin hæmolysis, and so small as to have no appreciable effect on the curve. The curve is, however, not quite symmetrical, but indicates a slight inhibition towards the end of the reaction; it has not been



thought permissible to stress this point, although it would be very convenient to do so, as the asymmetry is within the range of experimental error.

It is obvious that the measure of the skewness of the curve resulting from the differentiation of the curve obtained by experiment, describing the relation between percentage hæmolysis and time, will be a measure of any inhibition or acceleration occurring during the reaction. This and other problems will be considered in a subsequent paper.

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### *Recruitment Type of Reflexes.*

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In our previous papers\* comparison between isometric myograms of the ipsilateral knee flexor reflex and of the crossed knee-extensor reflex was, for the latter, practically confined to the decerebrate condition. We here supplement it by observations on the crossed knee-extensor reflex in the purely spinal preparation. The observations have been made after spinal transection in the 1st lumbar segment at times varying between two and nine days subsequent to the transection. The severance of the cord has been performed under deep anæsthesia and with full precautions for asepsis. At the subsequent examination of the reflexes the animal (cat) was decerebrated under profound anæsthesia. The myograph was, as before, isometric. The characteristics attaching to the crossed extensor's reflex, as described in our previous papers, we find largely obtain in the spinal preparation also. The ascent curve of the reflex has the long climbing course (fig. 1, A, B, C). Its

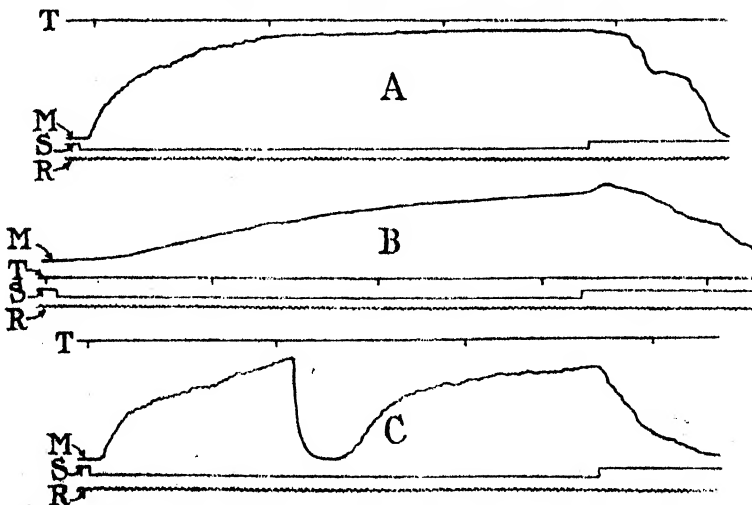


FIG. 1.—Spinal preparation. Reflex contraction of *vastocruureus* from contralateral popliteal n.; isometric records. A, 4 days after transection at 1st lumbar level; stimulus rate 32 per sec. B, 2 days after transection at 1st lumbar level; stimulus rate 30 per sec. C, similar to A, but with intercurrent inhibition by a single break-shock stimulus to ipsilateral popliteal nerve; contral. n. stimulation at 20 per sec. T, time in seconds; M, myograph; S, stimulation signal; R, stimulus rate.

\* These 'Proceedings,' B, vol. 95, p. 142; *ibid.*, p. 299 (1923).

duration has, in these experiments, varied between 1.3 seconds and 9 seconds, and in the latter instance the stimulus was finally withdrawn while the ascent was still in progress. In one experiment (4th day subsequent to spinal transection) the form of the ascent resembled somewhat that shown in fig. 12 E of the previous paper,\* there taken from a decerebrate preparation. In other experiments the ascent began less steeply and was of longer continuance, resembling the sub-variety D of fig. 12 of the decerebrate records. In this form the contraction tended to commence with a single small step followed by a slight decline before entering, perhaps a third of a second later, on its gradual continuous course of ascent.

Besides the characteristic slow prolonged ascent the reflex in its spinal form likewise presents relative insensibility to omission of a stimulus from the series exciting the afferent nerve, a feature noted in the decerebrate condition. Thus the suppression, during the course of the reflex, of one member of a series of break-shocks at 28 per second caused no remission whatever of the tension obtaining in the myogram, resembling in so far the result instanced in fig. 9† of a previous paper illustrating the decerebrate reaction.

The lumbo-sacral region of the spinal cord can therefore of itself in isolation from other spinal and prespinal centres develop the process answerable for the slow cumulative progress of the tetanic contraction of this reflex, and for its "momentum" character. In these features the crossed extensor knee-reflex contrasts with the ipsilateral spinal flexor reflexes and, indeed, with the ipsilateral reflex of the knee-extensor itself.

Yet in the spinal preparation the isometric myograms of the crossed knee-extensor reflex present, beside the above similarities to the decerebrate, certain differences from them. Thus, for reflex tetani of equivalent tension there is required usually stronger stimulation of the afferent nerve in the spinal (lumbar) preparation than in the decerebrate. Even with strong stimuli the crossed knee-extensor tetani are weaker in the spinal preparation than those which can be evoked in the decerebrate. In some spinal preparations the crossed knee-extensor reflex is in our experience not elicitable. This conforms with the experience of T. Graham Brown‡ in regard to the gastrocnemius-soleus, also an extensor. "The extensor reflex, as evidenced in the muscles of the leg, is not seen as a regular phenomenon in the low spinal preparation," *i.e.*, is not obtainable with regularity. In several of our spinal

\* These 'Proceedings,' B, vol. 95, p. 320 (1923).

† *Ibid.*, p. 151.

‡ 'Quart. Jnl. of Expt. Physiol.,' vol. 5, p. 247 (1912).

preparations we observed that though the crossed knee-extensor reflex was quite weak, or even inelicitable, the knee-jerk was easily obtained, and the tension developed by a single knee-jerk, recorded by the same isometric myograph, attained sometimes more than double the maximal tension obtainable by the most prolonged crossed extensor-reflex tetanus. The marked difference between the ipsilateral reflex and contralateral reflex of the knee-extensor we adverted to previously. Not rarely the tetani of the crossed extensor-reflex are less strong under strong stimulation of the afferent nerve than under weaker; there then ensues a terminal increase of contraction on cessation of the stimulus (fig. 1B), significantly suggesting that inhibition is admixed with excitation in the reflex effect. This terminal increase is greater after stronger stimuli than after weaker. This is seen both with spinal and decerebrate preparations, but especially with the former. This terminal increase of contraction ensuing on cessation of the stimulus is Graham Brown's terminal "extensor rebound contraction after excitation,"\* found by him in the gastrocnemius, both spinal and decerebrate.

But apart from this terminal rebound, a difference in our experience between the spinal and the decerebrate form of the crossed knee-extensor reflex lies in the terminal after-discharge in the former being less than in the latter. In the former the after-discharge is maintained less long at plateau-height, that is, the after-discharge of "total" character, in the sense described in our previous paper, lasts less long. Its rate of subsidence is also quicker and is subject to irregular waves. Connected with this shorter maintenance of after-discharge at plateau level may be the somewhat lesser degree of obliteration, in our experience, of the stimulus rhythm in the spinal than in the decerebrate examples of the reflex. Tremor synchronous with a stimulus rhythm of 30 per second has in two of our "low" spinal experiments been more marked than is usual at that frequency in the decerebrate preparation. But in the spinal as in the decerebrate preparation the degree of tremor at 30 per cent. stimuli was much greater in the ipsilateral extensor reflex than in the contralateral reflex of the same muscle in the same experiment.

How low the frequency of the stimulus may be in the decerebrate preparation before a corresponding tensile rhythm appears in the crossed knee-extensor reflex is shown by the contractile rhythm being in some cases not evident even with a stimulus rate of 16 per second. Fig. 2A shows, from such an experiment, the reflex under stimuli at 11 per second, giving even then only slight evidence of the stimulus rhythm, although very clearly rhythmic on

\* 'Quart. Jnl. of Expt. Physiol.,' vol. 4, p. 346 (1911).

further reduction of the stimulus rate to 7 per second (fig. 2B). Under a stimulus rate of 11 per second, intercurrent suppression of three successive

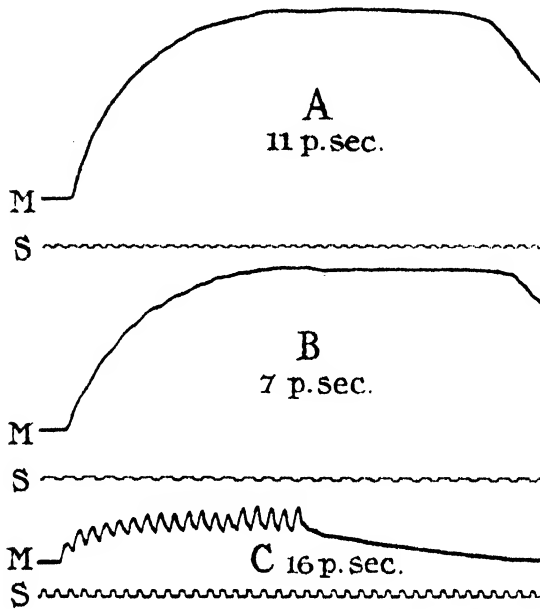


FIG. 2.—A and B. Reflex contraction of *vastocrureus* from contralateral popliteal n.; decerebrate preparation. In A stimulation rate 11 per sec.; in B stimulation rate 7 per sec.; in each case prolonged terminal after discharge at plateau level. C. Reflex contraction of *semitendinosus* from ipsilateral popliteal n.; stimulation rate 16 per second; spinal preparation. Isometric records. M, myograph; S, stimulation rate.

stimuli during the reflex tetanus ascent induced no drop in the contraction but merely a horizontal hiatus in the ascent; on recontinuance of the stimuli after their interruption for 0.3 seconds, the development of contraction tension recommenced as from the point already reached before the interruption. All this contrasts strongly with the knee-flexor reflex (ipsilateral spinal); fig. 2C illustrates this in *semitendinosus*, showing the great incompleteness of the reflex tetanus under stimuli at 16 per second as compared with the slight incompleteness of the tetanus of the extensor reflex at 7 per second.

In the spinal preparation the reaction (fig. 1C) of the crossed knee-extensor reflex to the inhibitory effect of a single strong break-shock applied to the ipsilateral popliteal nerve resembles closely that obtained in the decerebrate preparation. It will be seen that the inhibition can be total, and that after it the recovery of the ascent may show a sigmoid form, perhaps due to the

inhibitory effect persisting longer for some of the extensor moto-neurones than for others of them. In the purely spinal crossed extensor reflex we have so far not met with the markedly sigmoid form of initial development of the reflex frequent in decerebrate preparations.

The gradual cumulative onset of the isometric myogram in the crossed knee-extensor reflex is therefore present when the reflex is purely spinal as well as when it is decerebrate. Prespinal tonic centres are therefore not essential for what we term the "recruitment," although in the decerebrate preparation they influence it. That the recruitment is traceable to possible tonic influences exerted *via* the sympathetic is unlikely, in view of the seat of spinal severance for our spinal preparations having lain as far back as the first lumbar segment.

The ipsilateral spinal reflexes of knee-flexor, knee-extensor and ankle-flexor examined in our previous paper contrast therefore with those of crossed knee-extensor, both spinal and decerebrate, the former being *réactions d'emblée*, resembling in so far mn. tetani; the latter reflexes evidencing what we interpret as "recruitment." Recruitment or its absence appears thus to be a character distinctive of reflex groups. There is evidence that it extends to other reflexes beyond those above cited. The ipsilateral spinal hip-flexor reflex (*tensor fasciæ femoris*), as judged from records of imperfectly isometric kind, yields tetani of the "réaction d'emblée type";\* indications of "recruitment" are *per contra* evident in records, similarly imperfectly isometric, not only from crossed decerebrate *vasto-crureus*,† but also from crossed spinal *gastrocnemius*.‡ As regards the ankle extensor *gastrocnemius-soleus*, both decerebrate and spinal, there exist the very extensive systematic records of Graham Brown. Their graphic method was isotonic rather than isometric, but they seem to us indubitably interpretable as evidencing what we should term "recruitment" both in the spinal and decerebrate condition, and for the ipsilateral as well as for the crossed reflex.§ The ipsilateral reflex in the ankle-extensor would thus differ from, so far as our experience goes, the analogous reflex in the knee-extensor. That recruitment obtains also for the crossed reflex of the shoulder-extensor, *supra spinatus*, in the decerebrate condition we judge from previous records of our own, obtained it is true without rigid isometry,

\* 'Roy. Soc. Proc.' B, vol. 83, p. 441 (1910).

† *Ibid.*, p. 443, fig. 7 (1911).

‡ 'Roy. Soc. Proc.' B, vol. 79, p. 338 (1907).

§ T. Graham Brown, 'Quart. Journ. of Expt. Physiology,' vol. 4, p. 364, fig. 15; p. 368, fig. 18 (1911); vol. 5, p. 249, fig. 8, p. 252, fig. 11; *see also* especially pp. 247-249 (1912).

i.e., with a thick short rubber resistance allowing some shortening of the muscle though not of isotonic freedom, and with the muscle-pull quite close to the axis of the lever. The contraction curves so given (e.g., fig. 3), are

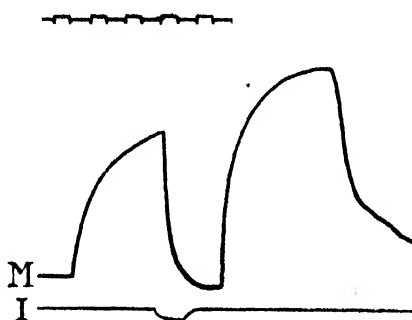


FIG. 3.—Decerebrate preparation. Reflex contraction of *supra-spinatus* (extensor of shoulder) from contralateral median n.; intercurrent inhibition by brief faradisation of ipsilateral median n. Record imperfectly isometric. M, myograph; C, stimulation of contralateral nerve; I, stimulation of ipsilateral nerve; T, time in seconds.

clearly of the “recruitment” type, and the intercurrent inhibitory relaxation and post-inhibitory reascent of contraction both resemble the crossed knee-extensor reflex.

Putting these data together the reaction “*d'emblée*” type of reflex includes :—

spinal ipsilateral hip-flexor, e.g., *tensor fasciæ femoris*, spinal ipsilateral knee-flexor, e.g., *semitendinosus*, spinal ipsilateral ankle-flexor, e.g., *tibialis anticus*, decerebrate ipsilateral knee-extensor, e.g., *vasto-crureus*.

The “recruitment” type of reflex includes :—

decerebrate contralateral knee-extensor, e.g., *rectus femoris*, and *vasto-crureus*, decerebrate contralateral and ipsilateral ankle-extensor, e.g., *gastrocnemius*, decerebrate contralateral shoulder-extensor, e.g., *supra-spinatus*, spinal contralateral knee-extensor, e.g., *rectus femoris* and *vasto-crureus*, and spinal contralateral and ipsilateral ankle-extensor, e.g., *gastrocnemius*.

*Observations on the Adjustment of the Human Body to  
Muscular Work.\**

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*Introduction.*—It has long been known that the dyspnœa produced by strenuous exercise, such as running or rowing, disappears if the work is continued and is replaced by a sense of great relief, the so-called "second wind." A certain time is necessary for the adjustment or accommodation which produces this sensation ; short-distance runners do not experience it, but those who are accustomed to long runs over the same course can predict at which lap or point they will obtain the relief of second wind. There are also individual differences ; in some men the sensation is very definite, in others so indefinite that it is unrecognised.

The observations of Cook and Pembrey† showed that during dyspnœa the percentage of carbon dioxide in the alveolar air was raised above the resting value and the respiratory quotient was unity or above unity, but during the hyperpnœa following the onset of second wind the percentage of carbon dioxide and the respiratory quotient fell ; the amount of air breathed per minute was less during hyperpnœa than during dyspnœa ; the rectal temperature showed a rise of about 1° F. during second wind, and as a rule there was a close association between the onset of sweating and second wind. Carbon dioxide appeared to be the chief factor in the adjustment of the respiratory and circulatory systems to the demands of the muscles for an adequate supply of blood.

The present series of observations was started with the hope of tracing more exactly the means whereby the adjustment was effected. The chief extension of the investigation was the determination of changes in the volume, acidity, ammonia and total nitrogen of the urine.

*Respiration (alveolar air).*—As in the previous research a slight modification

\* The expenses of this research were defrayed by a grant from the Royal Society. Preliminary communications were given to the Physiological Society, March, 1921, and Oct., 1922.

† 'Journ. Physiol.,' vol. 45, p. 429 (1912-1913).



Subject.	Rest.			Dyspnoea.			Second wind (onset).			Further exercise.		
	Date, 1921.	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub> / O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub> / O <sub>2</sub>	Distance and time: yds. & min.	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub> / O <sub>2</sub>	Distance and time: yds. & min.
N.W.M.	8.ii.	4.91	15.63	0.90	6.79	12.9	0.81	495 in 2½	6.37	13.35	0.79	495 in 2½
	16.ii.	5.61	15.65	1.06	6.45	14.39	0.98	495 in 2½	6.24	13.90	0.87	1210 in 8½
	20.ii.	4.65	—	—	6.49	—	—	550 in 2½	5.79	—	—	660 in 7½
	25.v.	4.62	—	—	6.26	—	—	495 in 2½	6.18	—	—	3020 in 16
	Average	4.95 (38 mm.)	15.64	—	6.50 (49 mm.)	13.65	—	—	6.15 (47 mm.)	13.62	—	715 in 9
E.C.W.	4.ii.	5.97	13.56	0.77	6.38	15.31	1.14	365 in 2	5.71	14.8	0.91	550 in 2½
	18.ii.	5.51	14.28	0.79	5.81	15.33	1.04	550 in 2½	5.92	15.46	1.11	605 in 3½
	21.ii.	5.46	—	—	—	—	—	605 in 3½	5.06	—	—	660 in 4
	Average	5.65 (43 mm.)	13.92	—	6.10 (46 mm.)	15.32	—	—	5.56 (43 mm.)	15.13	—	—
H.J.W.J.W.	23.ii.	5.71	—	—	6.13	—	—	330 in 1½	6.04	—	—	385 in 3
	25.ii.	5.80	—	—	6.06	—	—	330 in 1½	4.65	—	—	550 in 3
	Average	5.76 (44 mm.)	—	—	6.10 (46 mm.)	—	—	—	5.35 (41 mm.)	—	—	—
W.R.S.	1.ii.	4.15	17.02	1.08	5.43	16.39	1.25	330 in 1½	4.91	16.13	1.03	385 in 2
	15.ii.	4.56	15.71	0.84	5.01	16.14	1.05	495 in 1½	5.27	15.39	0.94	605 in 2
	Average	4.37 (33 mm.)	16.37	—	5.22 (40 mm.)	16.27	—	—	5.09 (39 mm.)	15.76	—	—

was made in the method of Haldane and Priestley\* for the collection of the alveolar air; the gas-sampler was made vacuous by a mercury pump, and the last portion of an adequate expiration was collected, in order that no abnormality should arise from delay or undue interference with the breathing after exercise. The running was at the average rate of  $7\frac{1}{4}$  miles per hour, but was rendered more strenuous by the fact that there were in the mile 32 laps with 128 turns at a right angle.

An examination of Table I shows that the results are not uniform throughout. This is to be attributed to the difficulty or impossibility of ensuring that the samples are taken under similar conditions of pulmonary ventilation, when the subject has been running rapidly. The results, especially the averages, confirm those previously obtained, namely, a rise of carbon dioxide during dyspnoea and a fall during second wind. In order to extend the evidence upon these points experiments were made upon the volume and composition of the air expired during rest, dyspnoea and second wind. The results are shown in Table II.

It will be seen from these data that the pulmonary ventilation is reduced, in some cases greatly, on the onset of second wind, as in the experiments of Cook and Pembrey. It is of interest to note that the volume of air breathed per minute was almost constant throughout the run of  $1\frac{1}{2}$  miles on the occasion when the subject did not experience second wind and complained of "stitch." The analyses of the expired air, which was collected during a pause of half a minute at each stage, show a rise in the percentage of carbon dioxide during dyspnoea; this was reduced during second wind. The average output of carbon dioxide during dyspnoea was 14.5, and that during second wind 10.7 times the resting value; the corresponding increases in the absorption of oxygen were 10.7 and 8.5.

The sense of discomfort during dyspnoea is associated with increased pulmonary ventilation, the sense of relief at the onset of second wind with diminished ventilation. According to the law of excitation the sensation would depend not upon the amount of change alone, but the amount in relation to the starting value and the time in which the change occurred. In order to test this, a series of graduated exercises was taken, ranging through 2.7, 3.6, 5.5 and 7.3 miles per hour, and the pulmonary ventilation was determined by samples taken at intervals of about five minutes. Apart from the interruptions for the half-minute samples, the walking and running were continuous. Under such conditions there was no definite sensation of second wind. The alterations in speed were made suddenly at a known point; the results indicate

\* 'Journ. Physiol.,' vol. 32, p. 226 (1905).

# Ta Pulmonary Ventilation of Men and y after Exercise

Subject.	Date, 1921.	Rest.	Dyspnoea.		Second wind (onset).		Further exercise.	
		Volume expired litres per min.	Distance in yards.	Volume expired litres per min. at 15° approx.	Distance in yards.	Volume expired litres per min. at 15° approx.	Distance in yards.	Volume expired litres per min. at 15° approx.
E.C.W.	14.ix.	7.5	330	45.0	935*	38.7	1320*	36.5
		7.3						
		7.5						
H.J.W.J.W.	14.ix.	10.4	550* 330	46.6	935	50.2	1270	49.4
		9.95						
		11.4						
		12.2						
		9.9						
N.W.M.	15.ix.	8.8	275	64.8	1540	41.9	1920 2360 2920 3300	40.8 35.1 33.4 35.4
		7.9						
		6.9						
		8.4						
		7.9						
	14.ix.	10.4	330	45.5	No second wind experienced by subject, who complained of "stitch" throughout the run.	52.2 50.8 51.3 48.5		
		9.1						
		9.7						
		9.0						
		8.6						
		9.6	605	50.4				
		10.1						
		8.0						
		9.8						
		9.9						

E.C.W.	21.xi.	6.5 7.0 $\text{CO}_2 = 3.89 \text{ p.c.}$ $= 272 \text{ c.c.}$ per min.	495 in $2\frac{1}{4}$ min.	54.5 $\text{CO}_2 = 4.58 \text{ p.c.}$ $= 2496 \text{ c.c.}$ per min.	1265 in $3\frac{1}{10}$ min.	41.4 $\text{CO}_2 = 4.13 \text{ p.c.}$ $= 1710 \text{ c.c.}$ per min.	2145	5.5 4.43 p.c. 2016 c.c. per min
		8.3 $\text{CO}_2 = 2.4 \text{ p.c.}$ $= 199 \text{ c.c.}$ per min. $\text{O}_2 = 17.53 \text{ p.c.}$ $= 284 \text{ c.c.}$ per min.	385	56.0 $\text{CO}_2 = 4.68 \text{ p.c.}$ $= 2620 \text{ c.c.}$ per min. $\text{O}_2 = 16.63 \text{ p.c.}$ $= 2430 \text{ c.c.}$ per min.	1100	44.5 $\text{CO}_2 = 4.21 \text{ p.c.}$ $= 1873 \text{ c.c.}$ per min. $\text{O}_2 = 16.07 \text{ p.c.}$ $= 2178 \text{ c.c.}$ per min.		
H.J.W.J.W.	23.xi.	$\text{CO}_2 = 0.65$ $\frac{\text{CO}_2}{\text{O}_2}$		$\text{CO}_2 = 1.11$ $\frac{\text{CO}_2}{\text{O}_2}$		$\text{CO}_2 = 0.83$ $\frac{\text{CO}_2}{\text{O}_2}$		
		5.5 $\text{CO}_2 = 2.27 \text{ p.c.}$ $= 125 \text{ c.c.}$ per min. $\text{O}_2 = 17.58 \text{ p.c.}$ $= 186 \text{ c.c.}$ per min.	330 in $1\frac{1}{4}$ min.	40.4 $\text{CO}_2 = 4.88 \text{ p.c.}$ $= 1975 \text{ c.c.}$ per min. $\text{O}_2 = 15.06 \text{ p.c.}$ $= 2380 \text{ c.c.}$ per min.	715	29.0 $\text{CO}_2 = 4.72 \text{ p.c.}$ $= 1368 \text{ c.c.}$ per min. $\text{O}_2 = 15.05 \text{ p.c.}$ $= 1712 \text{ c.c.}$ per min.		
N.W.M.	24.xi.	$\text{CO}_2 = 0.61$ $\frac{\text{CO}_2}{\text{O}_2}$		$\text{CO}_2 = 0.79$ $\frac{\text{CO}_2}{\text{O}_2}$		$\text{CO}_2 = 0.76$ $\frac{\text{CO}_2}{\text{O}_2}$		

\* The distances given are the totals starting from the beginning of the running.

that the ventilation of the lungs does not overshoot the mark at the later (the highest speeds) as it does at the earlier (the lower) speeds. Questions of bodily temperature and fatigue might come in here, for the total distance was about  $5\frac{1}{2}$  miles in 70 minutes, of which one-half was slow walking and one-half running, made more severe by frequent turns. These results must be compared with those obtained when the subjects started from the resting condition to take exercise at similar speeds, only one experiment at a definite speed being performed on one occasion. In each case there is a big increase in the pulmonary ventilation, followed in every case but one by a definite fall ; this is a contrast to the smaller changes seen when the subject started from a condition of previous exercise at a slower rate. To test further the application of the law of excitation to the sensation of second wind, an experiment was made in which a slow walk was carefully and gradually increased against the time of a stop-watch until the pace became a run at  $8\frac{1}{2}$  miles per hour ; no second wind was experienced and the increase in the pulmonary ventilation is represented by a gradually ascending curve, which in the case of the fittest subject (N.W.M.) is quite smooth and reaches a value of 61 litres per minute.

Second wind was not experienced at a rate of exercise below 3 laps per minute, that is, 5.5 miles per hour, and after previous exercise at a slower rate was not very definite even at a speed of 7.3 miles per hour. But starting from rest the subjects running at the last-mentioned speed noted a definite second wind associated with a considerable fall in the pulmonary ventilation. In two cases only of running at these higher speeds no relief was felt, and the fall in the ventilation was slight. These results are in accord with the law of excitation.

*Circulation.*—Elsewhere have been recorded observations\* upon the pulse of medical students after running a mile. The fittest men showed the greatest percentage rise in the pulse rate, and the greatest percentage fall toward the resting value. The active muscles have such a great need of arterial blood that the heart may be beating at a rate of 177 per minute at the end of the run. The oliguria and anuria, which will be described under the excretion of urine, appear to be due to constrictor impulses sent to the splanchnic area in order to increase the volume of blood circulating through the muscles. The experience of trainers of athletes, horses and dogs has shown the importance of meeting this demand of the muscles ; as examples may be mentioned the

\* Hambly, Hunt, Parker, Pembrey and Warner, 'Guy's Hospital Reports,' vol. 72, p. 367 (1922).

relation of meals to time of exercise, and scanty clothing, or clipping of the fur or selective breeding of thin coats in relation to the vascularity and temperature of the skin. The cooling of the skin is of value not only in the regulation of temperature, but in the constriction of the blood vessels for the maintenance of the pressure and supply of blood to the muscles. These questions will be considered in more detail in later sections of this paper.

*Excretion of Urine.*—The adjustment of the body to the increased output of carbon dioxide and moisture by the lungs during muscular work might be effected in part by alterations in the excretion of acid\* and water by the kidneys. In order to test this, attempts were made to collect the urine before, during and after exercise, but under ordinary conditions it was found impossible to pass urine directly after the standard runs. For this reason a diuretic, 560 c.c. of hot tea with or without 5 grains of caffeine,† was taken after the bladder had been emptied; and all the experiments, unless otherwise stated, relate to the excretion of urine after drinking this standard diuretic. As soon as the diuretic action was established the subject again emptied the bladder and started to run and the urine was collected at the height of dyspnœa, the onset of second wind, after a further run and during the subsequent period of rest.

*Volume of Urine.*—In the first place it is necessary to consider the possibility of expelling the urine completely from the bladder, for in several observations upon two subjects (H.J.W. and W.R.S.) no urine could be passed on pausing from the run (7 miles per hour) at the height of dyspnœa, although up to that point urine had been passed. When running was performed by H.J.W., N.W.M. and E.C.W. with the bladder partly full at the beginning of the experiment the urine could be voided at the height of dyspnœa or during second wind; moreover, the bladder appeared to be emptied completely at the end of the run, for during the subsequent period of rest the quantities collected showed no definite increase. This conclusion is confirmed by the fact that distension of the bladder would have been observed if the excretion had continued at the rapid rate produced by the diuretic, for it would have contained about 500 c.c. of urine. These results show that inability to expel urine from the bladder was not the cause of the anuria; the cause was lack of urine to expel.

There arises also the question of latency or lag in the excretion of urine and its expulsion by the bladder. This was tested by swallowing 10 grains of

\* The blood becomes more acid as the result of exercise. (Parsons, Parsons and Barcroft, *Proc. Physiol. Soc., Journ. Physiol.*, vol. 53, p. cx. 1919-20).

† In none of the experiments actually recorded in this paper was caffeine taken.

potassium iodide in a cupful of tea directly the diuretic effect of the tea was established; at rest the presence of the drug was detected within four minutes, during running within six minutes. This period obviously includes transference and absorption in the alimentary canal and is therefore an outside limit for the latency; this conclusion is confirmed by the following curve (fig. 1) for the discharge of urine during a period in which two short runs were taken. In this case the latency appears to be about three minutes.

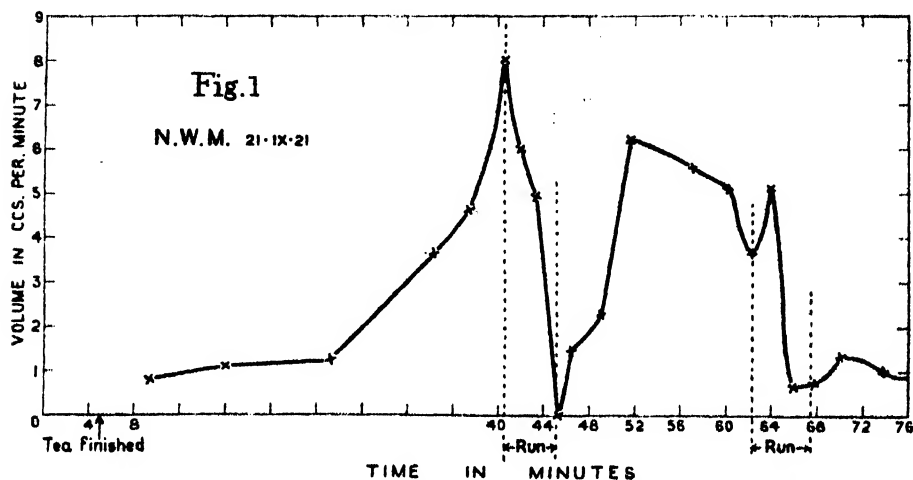


FIG. 1.—Volume of urine excreted by N.W.M. after 500 c.c. of hot tea. Urine was collected at short intervals during the runs at four laps per minute, in order to estimate the time between excretion and collection.

The actual results obtained for the excretion of urine during rest and the effects of running at the rate of 7.3 miles per hour will be shown best by the typical curves (fig. 2) and those relating to acidity (fig. 9).

In 12 experiments upon two subjects (E.C.W. and N.W.M.) the result was a rapid fall in the rate of the urinary excretion during the period of running until second wind was established, when the output had almost ceased, and no increase was observed until rest was taken; then a very rapid flow set in, followed by a rapid decline which was interrupted by a definite rise before the minimum was attained. This anuria, or oliguria, appeared as a constant feature during running. Experiments were made to determine the cause or causes of this condition. In the first place, comparisons have been made between the output before, during and after exercise and the output during rest, the quantity of the diuretic being the same.

The effect of running is a decrease in the total output of water by the kidneys.

The quantity discharged in other ways, the skin and lungs, must be estimated. This was done by weighing the subject in a very delicate balance before or

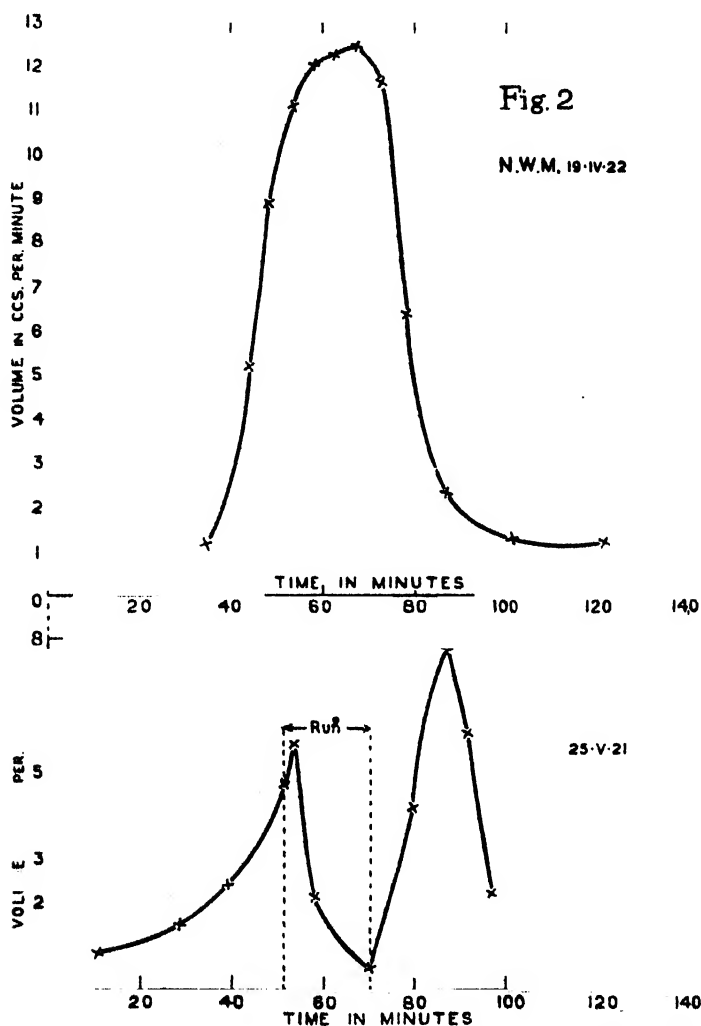


FIG. 2.—Volume of urine excreted by N.W.M. after 500 c.c. of hot tea. The top curve indicates the volume during rest, and the bottom curve that during running for 19 minutes at a rate of four laps per minute (7.28 m.p.h.). The first sample after the commencement of the run was taken when the subject was very dyspnoëic, the second sample directly second wind was experienced, and the third (at the end of the run) after the establishment of second wind.

directly after he had taken a known volume of liquid, before and directly after a run and again after a period of rest; the urine discharged was collected for



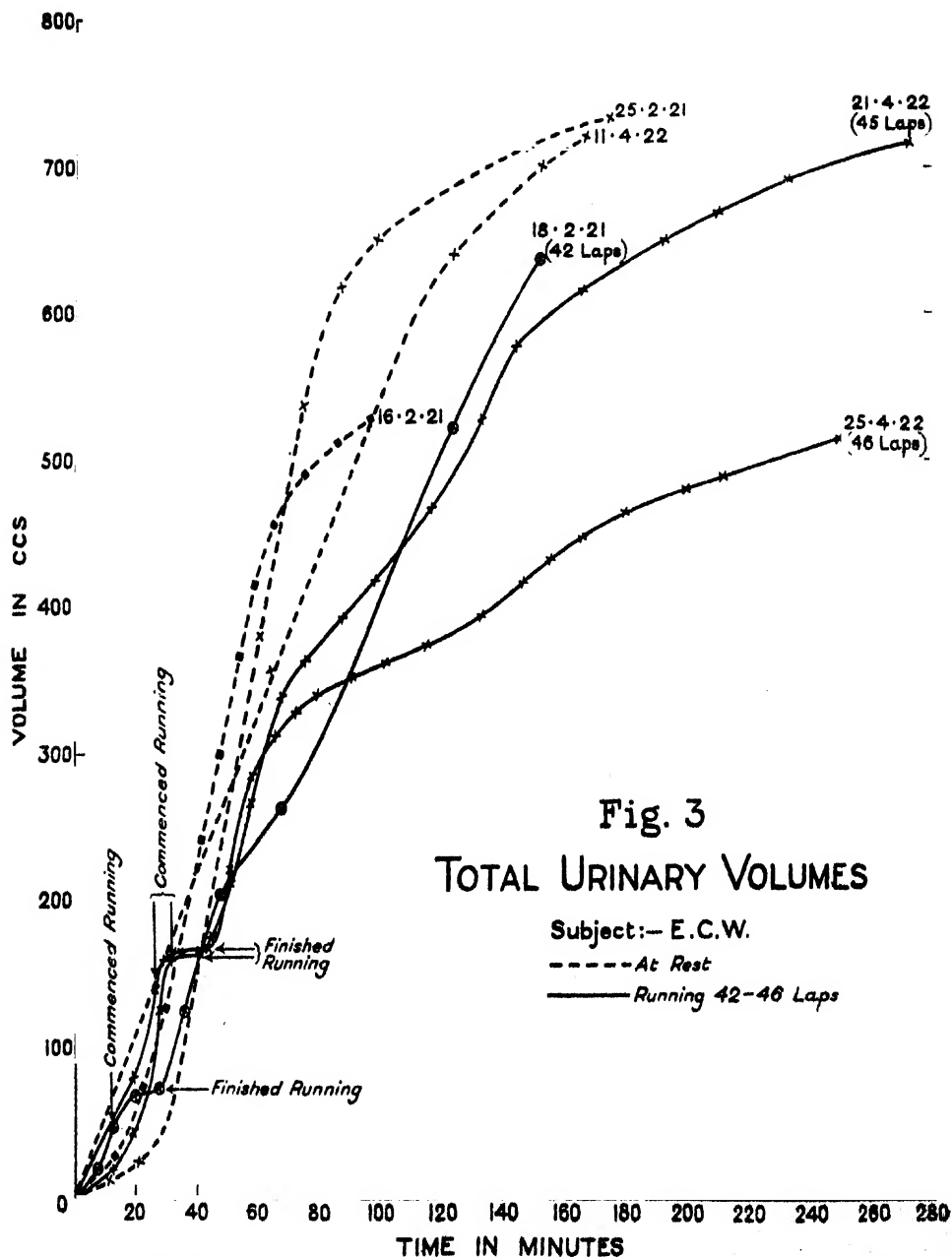


FIG. 3.—Volume of urine excreted by E.C.W. after 560 c.c. of hot tea, (a) rest, and (b) running at four laps per minute (7.28 m.p.h.).

the several periods. Zuntz\* has shown that the loss of body-weight is a rough estimate of the loss of water from the lungs and skin during a period in which there is neither intake of food nor output of urine and faeces. The error due to the gaseous exchange will slightly exaggerate this loss of weight, for a calculation from the analyses of the air expired during a run shows a loss of carbon dioxide discharged in excess of the oxygen absorbed of about 7 grms. in six minutes.

The general result is that the total loss of water under the influence of the diuretic was similar whether exercise was taken or not, but the loss by the skin and lungs was greater and that by the urine less when the run was performed. There was a conservation of water in the earlier stages of the run until sweating began; this indicates that the oliguria is not secondary to the increased loss by the skin and lungs. The ultimate result is the maintenance of the water-equilibrium of the body. If no diuretic had been taken evidence of disturbance of the equilibrium would have been present as a sensation of thirst after the exercise and sweating. In Table III is given a comparison of the total loss of water from the same subjects at rest and after exercise.

The anuria or oliguria observed during running is not due to any lack of water owing to the extra discharge by the skin and lungs, for as the curves in fig. 4 show, the total amount of moisture lost per minute during exercise of short duration is diminished, or during a longer run is delayed, as compared with the condition of rest. This receives confirmation from the fact that a run of only three laps (160 yards) is sufficient to produce a definite oliguria or even anuria (Fig. 9).

In order to investigate further the influence of muscular work upon the excretion of urine a series of experiments was made in which the exercise was graduated from a walk at three miles per hour up to a run at a speed of eight miles per hour; the exercise was continuous and started after the diuretic effect had been established, and during the experiment tea was taken from time to time in quantities sufficient to replace the water of the urine excreted. An examination of the curves (fig. 5) shows in each case an increase in the excretion during the period of walking up to the rate of five miles per hour, when the pace became a trot; at this stage the excretion in two cases fell rapidly towards zero, in the third case there was a slight rise and then a rapid fall to zero. During the subsequent rest the excretion rose to the original volume.

There are several possible causes of the rise in the urinary excretion

\* Zuntz u. Schumburg, 'Studien zu einer Physiologie des Marsches.' Berlin (1901).

Table III.

Rest.					Exercise.					
Date.	Time in mins.	Loss of water, c.c.			Date.	Time in mins.	Loss of water, c.c.			No. of laps.*
		Skin and lungs.	Urine.	Total.			Skin and lungs.	Urine.	Total.	
16.ii.21.	100	125†	530	655	21.iv.22.	100	366	424	790	45
25.ii.21.	"	125†	652	777	18.ii.21	"	—	390	—	42
11.iv.22.	"	93	540	633	25.iv.22.	"	340	360	700	46
25.ii.21.	150	187†	714	901	21.iv.22.	150	432	588	1020	45
11.iv.22.	"	139	695	834	18.ii.21.	"	—	631	—	42
					25.iv.22.	"	416	424	840	46
Subject = E.C.W.										
19.iv.22.	100	116	494	610	26.iv.22.	100	212	338	550	54
14.ii.22.	"	117	264	381	25.v.21.	"	—	320	—	58
	50	175	525	700	11.ii.21.	"	—	300	—	58
					9.iii.22.	"	—	302	—	8
					26.iv.22.	150	337	400	737	54
					9.iii.22.	"	—	488	—	8
Subject = N.W.M.										

32 laps = 1 mile.

† Calculated.

during the walk; for example, quickened circulation, constriction of the cutaneous vessels and a rise in the blood pressure. On the other hand, the oliguria or anuria during the run may be due to dilatation of the cutaneous vessels and constriction of the splanchnic vessels, including those of the kidney, for the maintenance of the blood pressure; to physico-chemical changes in the blood; or failure in the absorption from the bowels. Various attempts have been made to study these factors. The influence of the cutaneous circulation and sweating was investigated. Running stripped, with the exception of a loin cloth, at a temperature of  $10.5^{\circ}$  ( $50.9^{\circ}$ ) gave a similar curve, the decrease in the output of urine was produced by the exercise in spite of the constriction of the vessels of the skin and the diminished loss of water from the skin and, possibly, the lungs. Furthermore, a slight exercise, such as a slow trot at 3.64 miles per hour, in a cold room,  $12-15^{\circ}$  ( $53.6^{\circ}-59.0^{\circ}$ ) produced no definite alteration in the normal diuretic curve obtained during

rest, the subject being clothed in each case. Walking would have been a more economical method of progression, but the trot produced movements more closely resembling running.

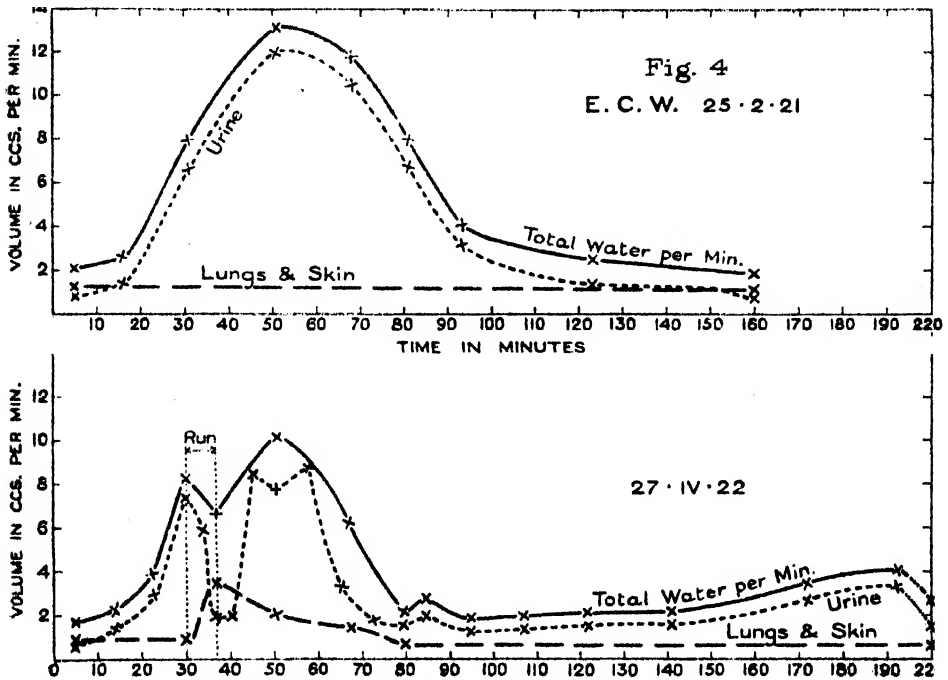


FIG. 4.—Loss of water by the skin and lungs and by the urine after 560 c.c. of hot tea had been taken by E.C.W. The top curve indicates loss during rest and the bottom curve the loss after a short run of 16 laps at a rate of four laps per minute (7.28 m.p.h.). The amount of water lost by skin and lungs during rest was calculated from the average of data obtained on other days.

The opposite effect on the cutaneous vessels and the loss of water by the skin and lungs was produced by exposure to a warm atmosphere ( $32.8^{\circ}$ ). Under such conditions there was a preliminary fall in the urinary output for three or four minutes on entering the warm room and starting to trot slowly at 3.64 miles per hour; this interruption of the normal diuretic curve appeared to be due to the dilatation of the cutaneous vessels; it was followed by a rise and a steady fall, which was broken for about 10 minutes by a slight rise on returning to the cold room and resting (fig. 6). This agrees with the recognised relation\* between the skin and the kidneys.

\* Gibson, 'Quart. Journ. Med.', vol. 3, p. 52 (1909-10); Debenham, Joffe and Pembrey, 'Guy's Hospital Reports,' vol. 73, p. 106 (1923).

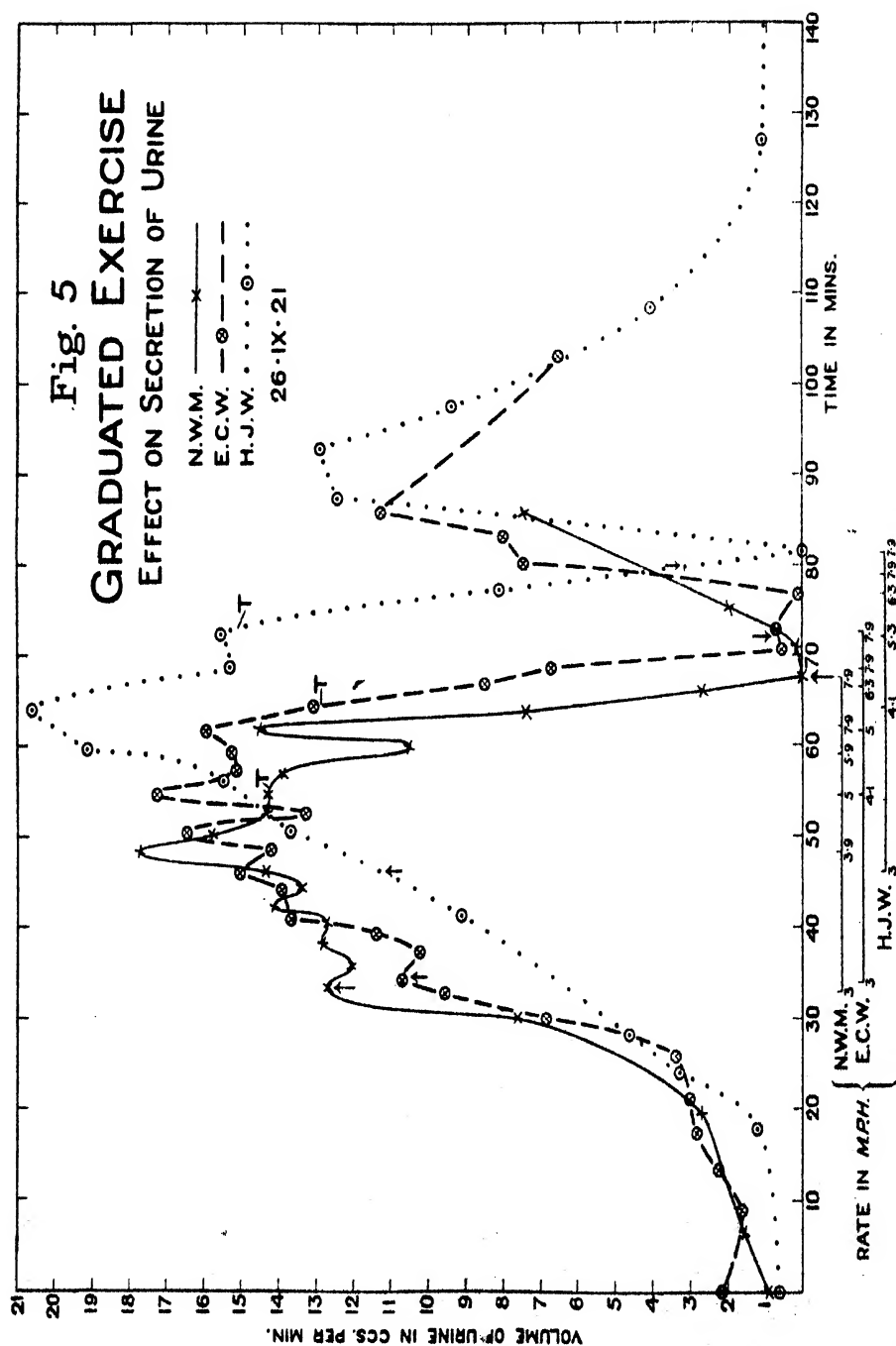


FIG. 5.—Volume of urine excreted by three subjects under the influence of the standard diuretic; warm tea was also taken from time to time to replace the water lost as urine. Gradually increasing exercise was performed; the beginning and end of the exercise are indicated by vertical arrows. The point at which the exercise became a trot is indicated by the letter T.

The excretion of urine was determined also when the subject remained for about 20 minutes in an electric bath, which left only the head free from the

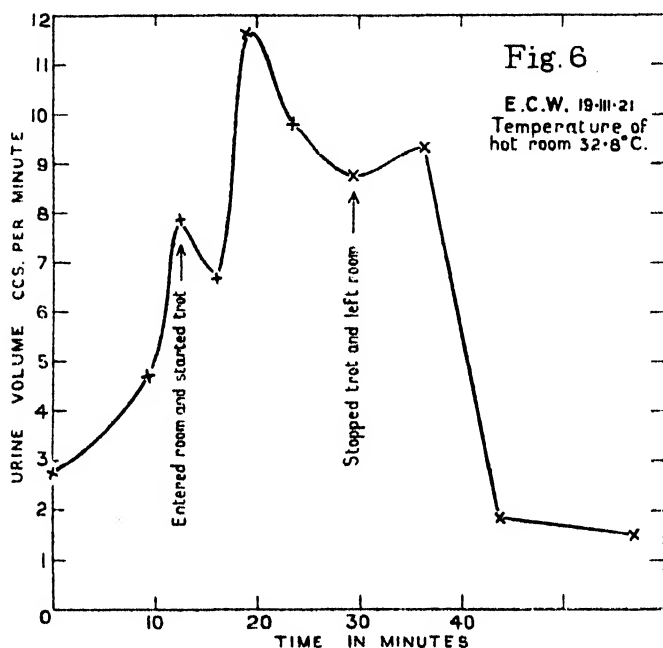


FIG. 6.—Volume of urine for E.C.W. after taking 560 c.c. of warm tea; trotting at a rate of about two laps per minute (3.64 m.p.h.) was performed in a hot room (32.8°), as indicated.

temperature of the air, 44.4° (112°) dry-bulb, 28.9° (84°) wet-bulb; although sweating and dilatation of the cutaneous vessels were pronounced, the diuretic curve resembled the normal at rest, but the quantity of urine was less. External temperature, therefore, does not affect materially the shape of the curve; some other cause of the oliguria during running must be sought.

It appears that some special effect is produced by strenuous exercise, such as running; there may be an overflow of nervous impulses or adrenaline which causes a constriction of the splanchnic area including the kidney. It has been shown by Krogh and others that on the intention to begin muscular work, the respiration, pulse and blood pressure are increased. On the three subjects of the present experiments a feigned run for three or four minutes, during which the tense attitude for starting to run was maintained, produced a definite decrease in the urine. It is also of interest that sweating was observed in two of the subjects. It is generally accepted that the secretory and motor activities of the alimentary canal are in abeyance during muscular

work, and it appears from the experiments here recorded that the kidneys also are involved in this inactivity.

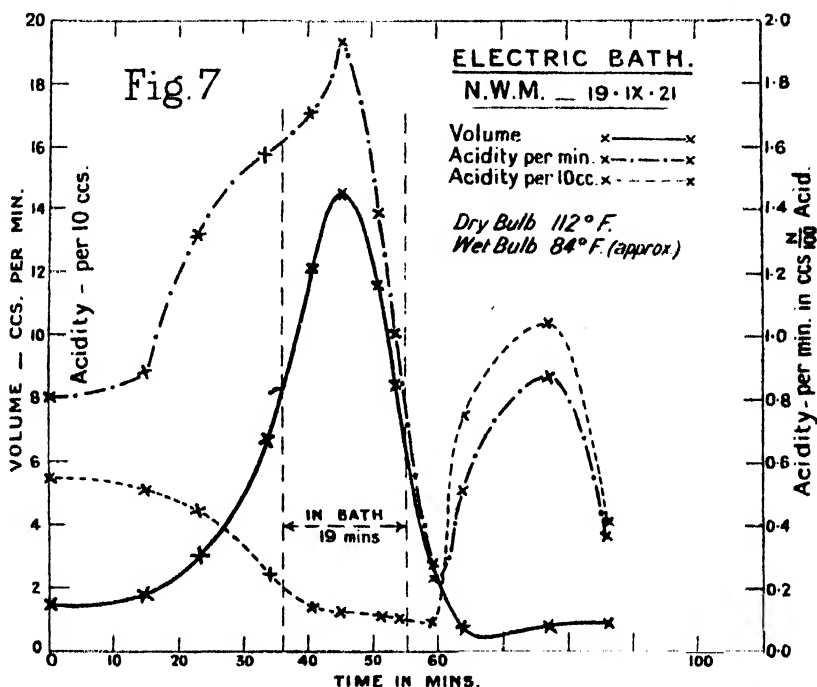


FIG. 7.—Volume and acidity of urine excreted by N.W.M. under the influence of the standard diuretic; the subject remained stripped in an electric bath during the period indicated between the vertical lines.

*Acidity of the Urine.*—The diminished or suspended excretion of urine during the hard muscular work might be advantageous by retaining water or acid or both; it is necessary, therefore, to know the volume and composition of the urine at different stages of a typical experiment. The respective samples of urine were collected under liquid paraffin and the acidity estimated on the same day by titration with N/100 NaOH to a Ph of 7.4, phenol red being used as the indicator (Walpole's method). In every case double titrations were made and sometimes by different observers; the collection of the samples was started in no case before 10.30 in the morning, for it had been found that specimens taken before this time were alkaline, as previously pointed out by Leathes.\* Apart from the standard diuretic nothing was taken during the experiment.

The following typical curves (figs. 8, 9) show the volume and acidity per

\* 'Brit. Med. Journ.,' vol. 2, p. 165 (1918).

minute of the urine passed at rest, during dyspnoea, at the onset of second wind and in the subsequent period, whether of further exercise or rest.

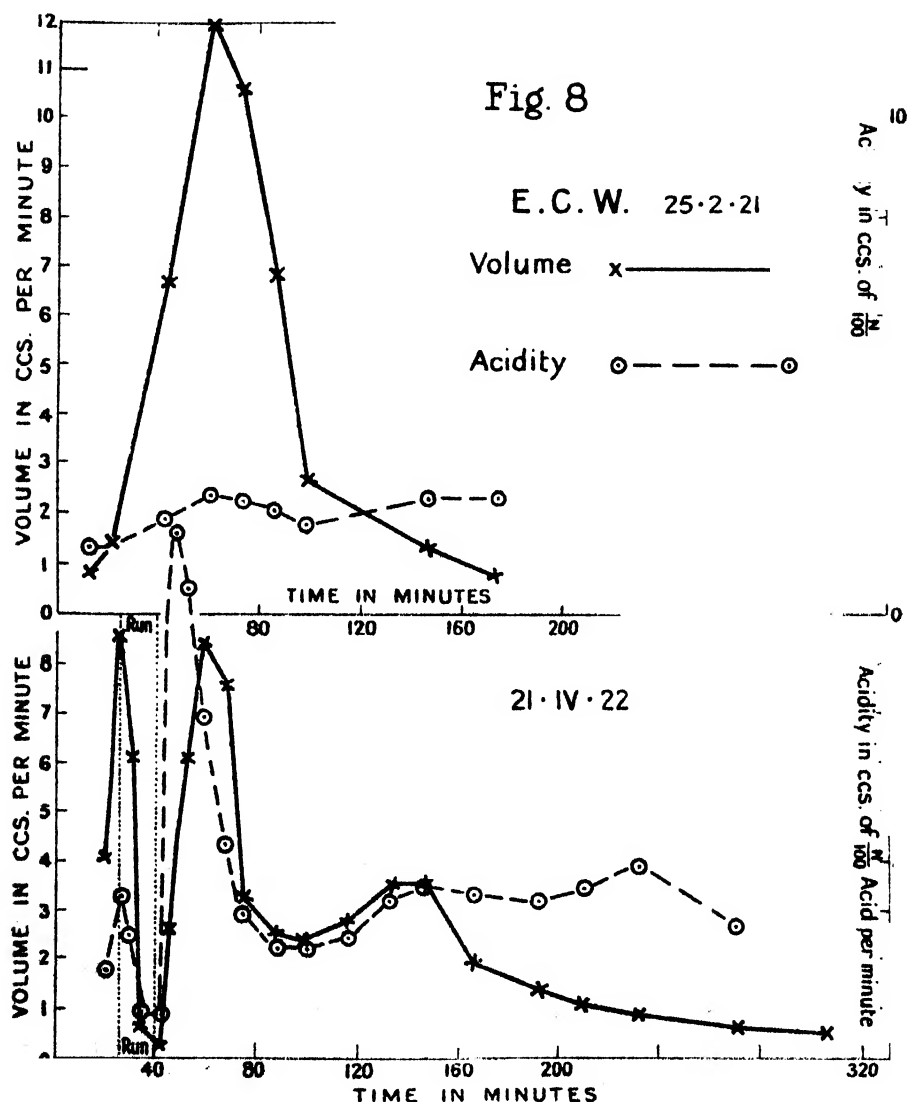


FIG. 8.—Volume and acidity of urine excreted by E.C.W. under the influence of the standard diuretic. The upper curve shows that excreted during rest, and the lower the results obtained when the subject ran 45 laps at a rate of four laps per minute (7·28 m.p.h.). The period of running is indicated by the vertical dotted lines. The first sample after running commenced was collected at the height of dyspnoea, the second directly second wind was experienced, and the third (at the end of running) after the sensation of second wind had been established.



The general course of the curve for acidity is similar to that for the volume both falling during dyspnoea ; but after the onset of second wind the acidity

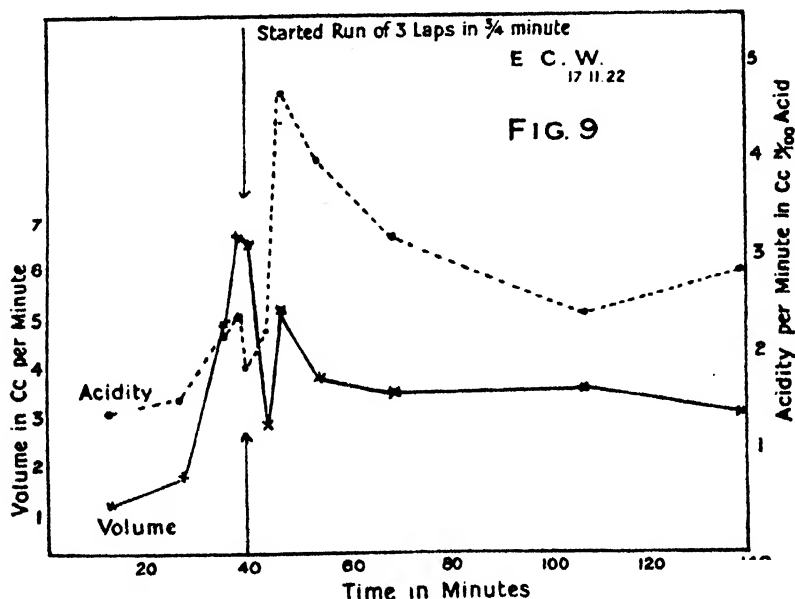


FIG. 9.—Volume and acidity of urine of E.C.W. (17.2.22) under the influence of the standard diuretic. Effect of a run of three laps (160 yards) at a rate of 7.6 miles per hour.

remains constant or rises, whereas the volume is still falling. On the cessation of exercise the acidity rises abruptly and reaches its maximum sooner than does the volume. In order to follow the activity of the kidney by signs other than the excretion of water the total output of nitrogen in the urine was determined by two Kjeldahl estimations of each sample. A consideration of the acidity in relation to the total output of urinary nitrogen is of value because the nitrogen may be considered in many respects as a measure of the renal activity.

The data show that during second wind the acidity-nitrogen ratio is about three to eight times as great as before the run, whereas during dyspnoea it is about the same. The total nitrogen falls during oliguria, but rises in the subsequent period of rest with the recovery of the output of water.

*Total Acid in the Urine.*—Compared with the normals at rest the curves for the total acid discharged in the urine show a delay during the run at 7.3 miles per hour and a compensation during the subsequent period of rest. When the subject walked or went at a slow trot the curve was very similar

to that obtained during rest, although there were individual differences in the case of the two subjects (E.C.W. and N.W.M.).

*Ammonia in the Urine.*—The data for the ammonia, estimated by a modification of Folin's method, give a fall during dyspnoea, a tendency to rise during second wind, and a definite rise in the subsequent period of running or rest. This condition was less marked in N.W.M. than in E.C.W., and in this respect there is agreement with the corresponding differences in the output of acid.

*Total Nitrogen in the Urine.*—This was estimated by Kjeldahl's method, and, as in the case of the other constituents, two estimations of each sample were made. The variations in the nitrogen follow those in the volume of the urine.

The ratio Ammonia Nitrogen/Total Nitrogen remains fairly steady during dyspnoea, but three or four minutes after the onset of second wind shows a pronounced rise.

*Chlorides in the Urine.*—In three or four experiments the chlorides in the urine were determined; the results agreed closely with the output of nitrogen before, during and after the oliguria produced by running.

*Albumin in the Urine.*—Several observers, Collier,\* Dunhill,† Leube and Zuntz, have found albumin in the urine passed by healthy men after strenuous exercise, such as boat-racing and marching with a load. The causes suggested are interference with the supply of blood to the kidneys, and the action of the metabolic products of severe muscular work. In the present research the urine was examined after three runs, but no albumin was found; the work was not severe or prolonged. When, however, sodium bicarbonate was taken before the exercise albumin was found in the urine excreted by W.R.S. after a run of 41 laps. A similar result was obtained in the experiments with ammonium chloride; in the sample of urine passed directly after a run of 48 laps by N.W.M., there was no albumin, but in that discharged 12 minutes later it was present and appeared in increasing quantity in the urine passed 46 minutes after the run; an hour or so later there was less. In the case of E.C.W., after taking a dose of ammonium chloride, a trace of albumin was found in one sample of urine passed during rest, but on another occasion a negative result was obtained; after the subject ran 42 laps, albumin appeared in the urine and increased in quantity for a few minutes later, to decrease within half an hour.

*Excretion of Water and Acid by the Skin.*—It has been mentioned that the

\* 'Brit. Med. Journ.,' vol. 1, p. 4 (1907).

† *Ibid.*, p. 1031.

onset of sweating closely corresponded with that of second wind. Experiments, therefore, were made to determine whether this method of accommodation to the effects of muscular work depended chiefly upon the evaporation of water or the excretion of acid. The results of the few experiments suggest that the relief from sweating during exercise is due chiefly to cooling by evaporation, for the concentration of lactic acid was found to be of the same order in sweat obtained while the subject was sitting in an electric bath.

The relation of the loss of water by the skin to the output of urine has been considered in detail in a previous part of this paper (*see p. 424*).

*The Effects of the Ingestion of (a) Sodium Bicarbonate and (b) Ammonium Chloride.*—In order to test the influence of an alkali, which has been advocated by some athletes, experiments were made after the ingestion of 10 grms. of sodium bicarbonate dissolved in the standard diuretic. During the run there was a rise in the percentage of carbon dioxide in the alveolar air, followed by a fall, but no sensation of second wind was experienced. In the first stages of this run the dyspnoea of a normal run was absent, but in the later stages there was a progressively increasing dyspnoea which eventually prevented the subject from proceeding. The pulmonary ventilation showed a rise throughout the exercise, and this supports the view that the relative fall in the ventilation during a normal run is responsible in great measure for the sensation of relief in second wind.

The results of these experiments are given in the curves opposite (*fig. 10*).

The methods of compensation employed by the body for the maintenance of its normal reaction are well shown, and a comparison of these curves with those for the controls during rest demonstrates the production of acid during muscular work. There was considerable sweating during the run, but the lactic acid was present in smaller concentration than in those cases in which no sodium bicarbonate was taken.

As a contrast to the effects of sodium bicarbonate, attempts were made to produce an experimental acidosis by taking large doses, 10 grms. of ammonium chloride dissolved in the standard diuretic. Nausea and discomfort were produced, and the sensations of the two subjects were conflicting. The curves for the acidity and the volume of the urine and the composition of the alveolar air at rest after a dose of ammonium chloride and the effects of running are given in *fig. 11*; again the results demonstrate clearly the means for the maintenance of the reaction of the body.

Briefly the effects of these two drugs may be stated as follows: During rest the sodium bicarbonate reduces the acidity of the blood, the respiratory centre

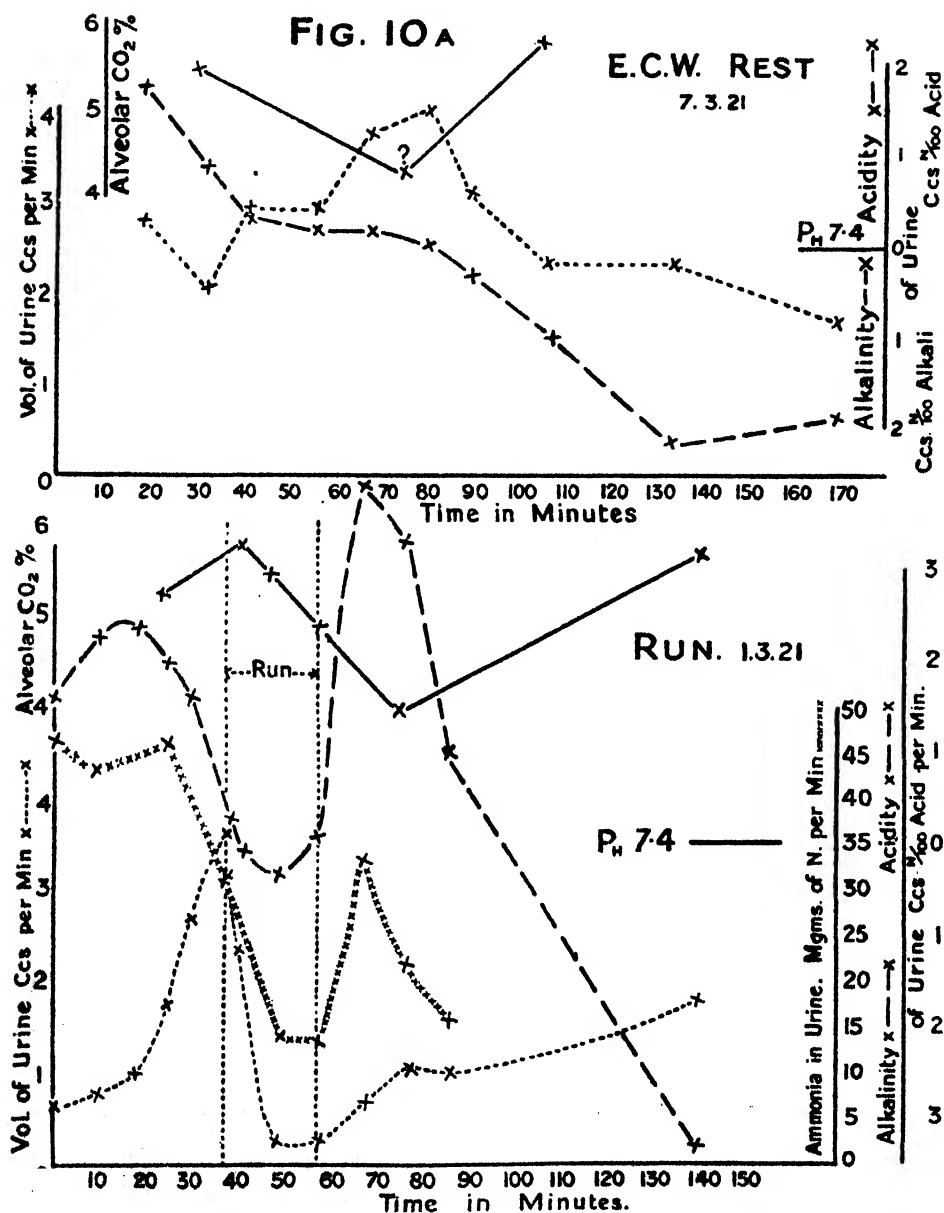
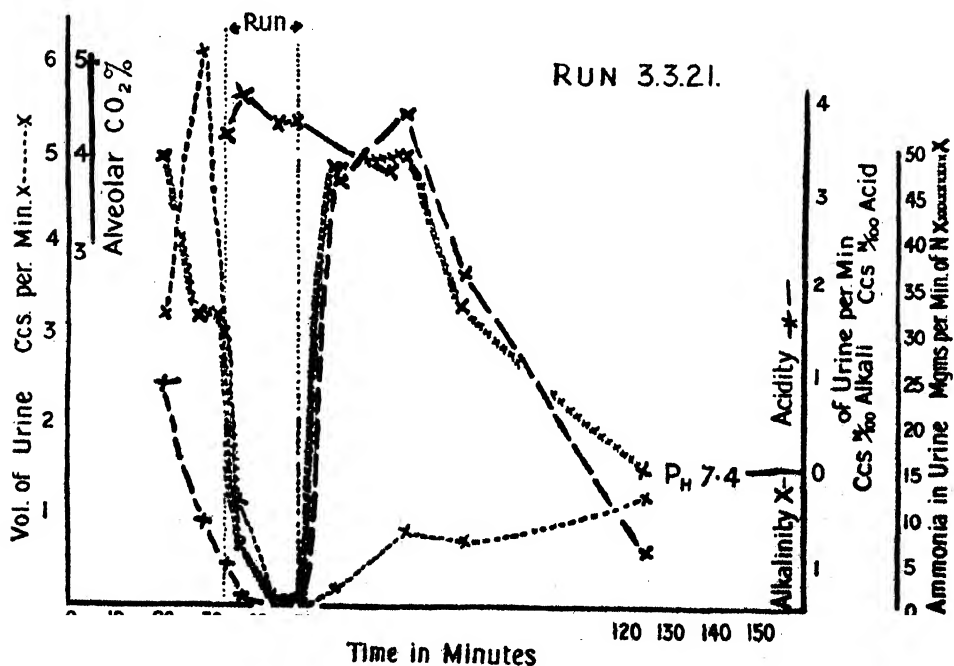
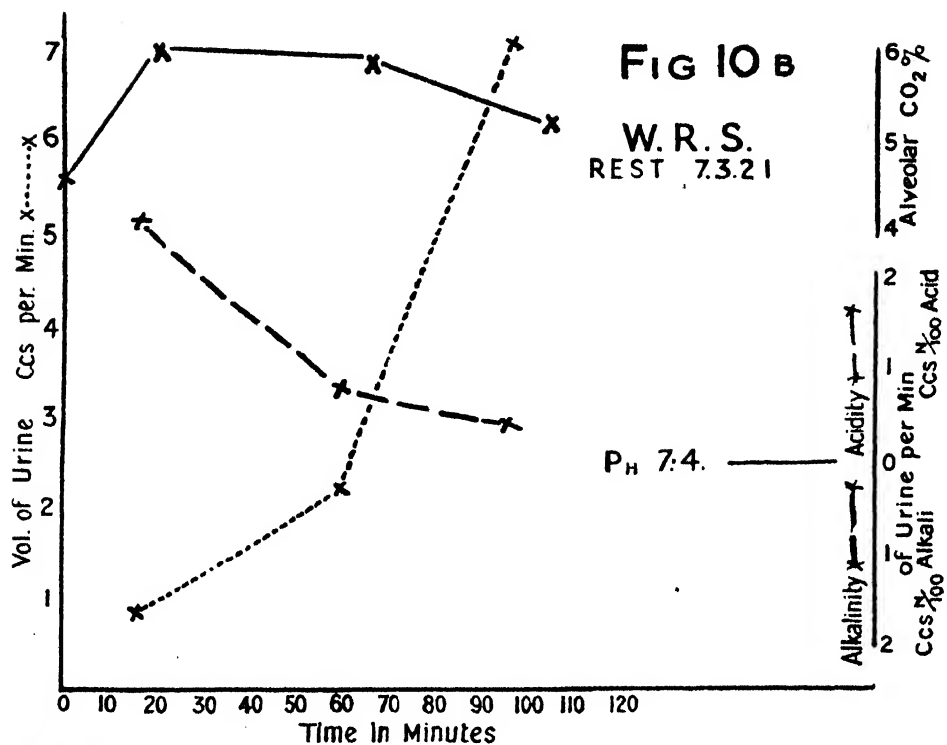


FIG. 10.—Volume, acidity and ammonia of urine and percentage of alveolar carbon dioxide for (A) E.C.W. and (B) W.R.S. after taking 560 c.c. of warm tea and 10 grms. of sodium bicarbonate. The top curve represents the results obtained during rest, and the bottom those during running at four laps per minute (7.28 m.p.h.) for the period included between the vertical lines. One value for carbon dioxide at rest was uncertain and is indicated by (?).



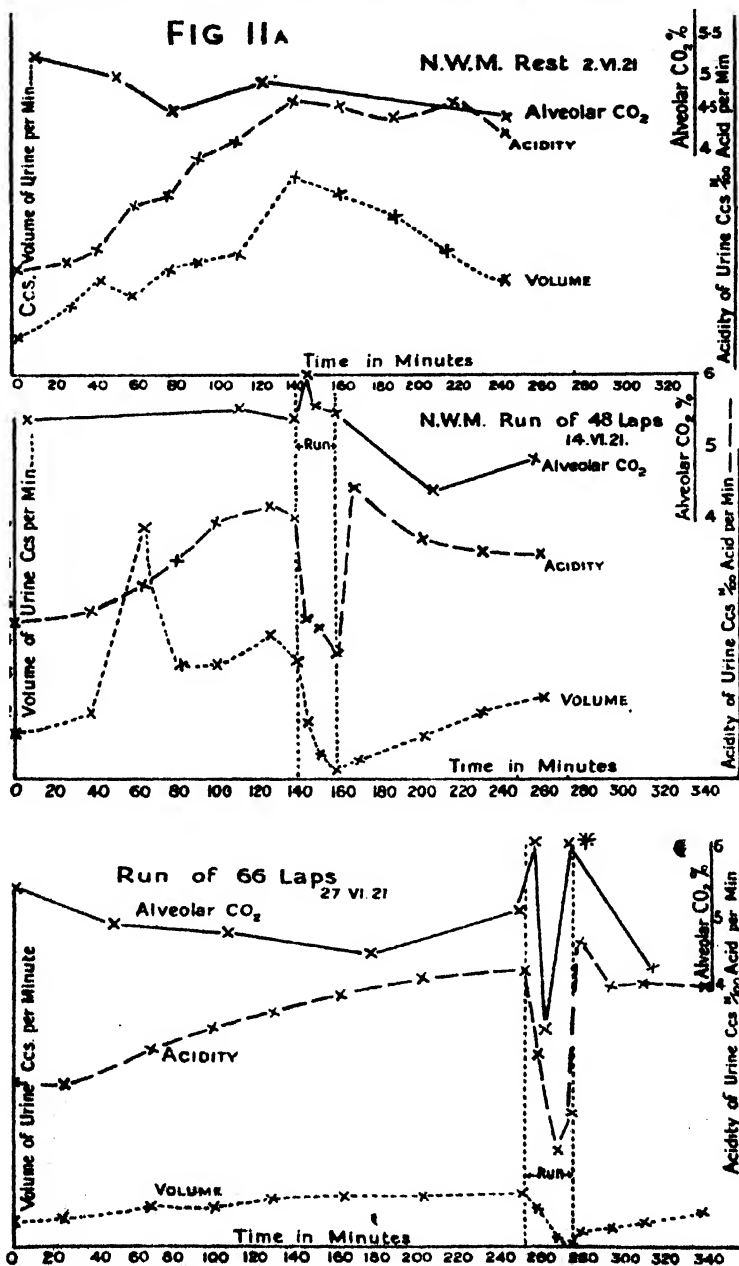


FIG. 11.—(A) Volume and acidity of urine and percentage of alveolar carbon dioxide of N.W.M. after taking 560 c.c. of warm tea and 10 grms. of ammonium chloride. The top curve represents the values obtained during rest, and the middle and bottom curves those found when the subject ran at the rate of four laps per minute (7.38 m.p.h.). The first sample after running commenced was taken at the height of dyspnoea, the second when second wind was experienced, and the third (at the end of running) after second wind had been established. The asterisk indicates the absence of "stitch."

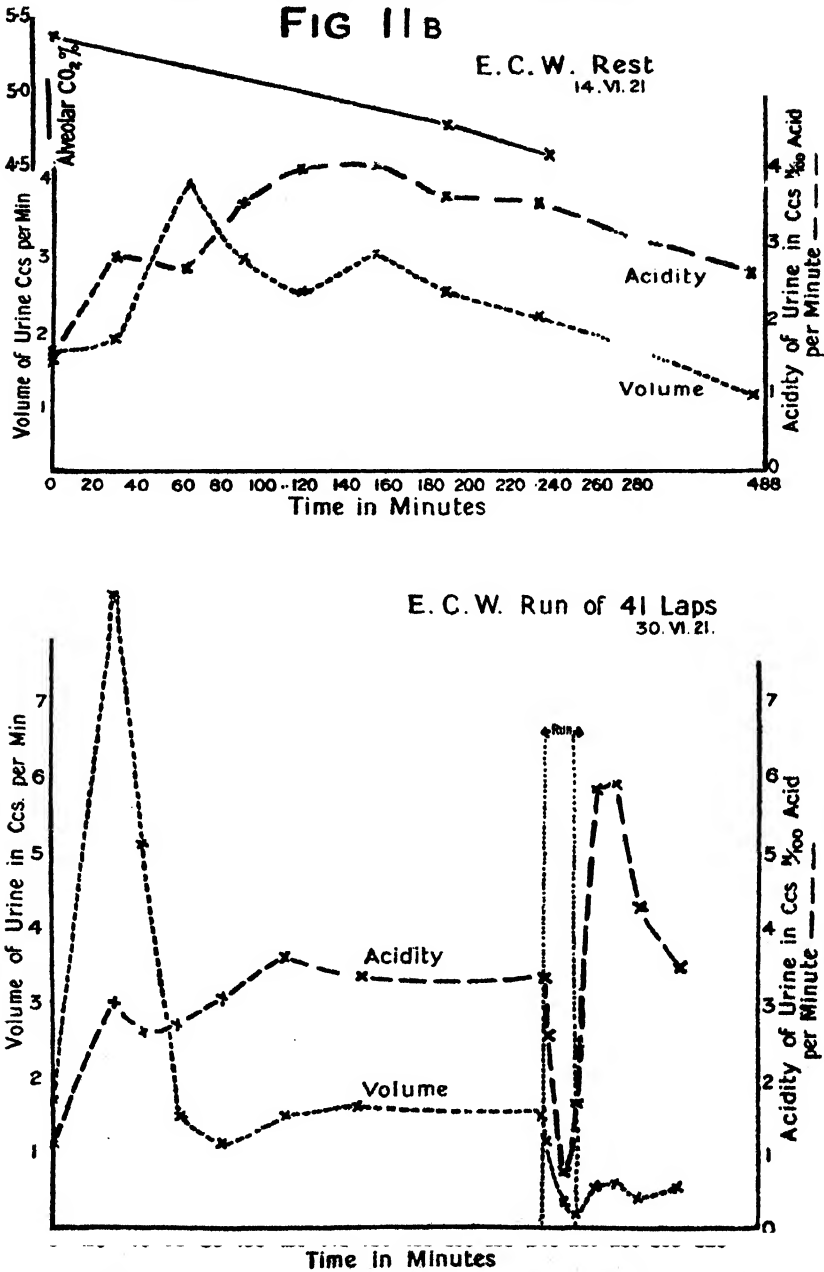


FIG. 11.—(B) Volume and acidity of urine and percentage of carbon dioxide in alveolar air of E.C.W. after taking 280 c.c. of warm tea and 8–10 grms. of ammonium chloride. The top curve represents the values obtained during rest, and the bottom those found when the subject ran at the rate of four laps per minute (7.28 m.p.h.). The first sample after running commenced was taken at the height of dyspnoea, the second when second wind was experienced and the third (at the end of running) after second wind had been established.

is not so readily stimulated, the pulmonary ventilation is diminished, the tension of the carbon dioxide in the alveolar air is raised and the urine becomes alkaline. On the other hand, the results are the exact opposite after a dose of ammonium chloride. During exercise after taking sodium bicarbonate the respiratory centre is not quickly stimulated by the acid metabolites, the increase in the pulmonary ventilation is delayed, there is no sudden change, no second wind but increasing dyspnoea and a prolonged rise in the ventilation. With ammonium chloride the ventilation is increased quickly and falls rapidly, and there is early sweating. As J. B. S. Haldane\* has shown, large doses of this drug will produce breathlessness at rest or on very slight exertion.

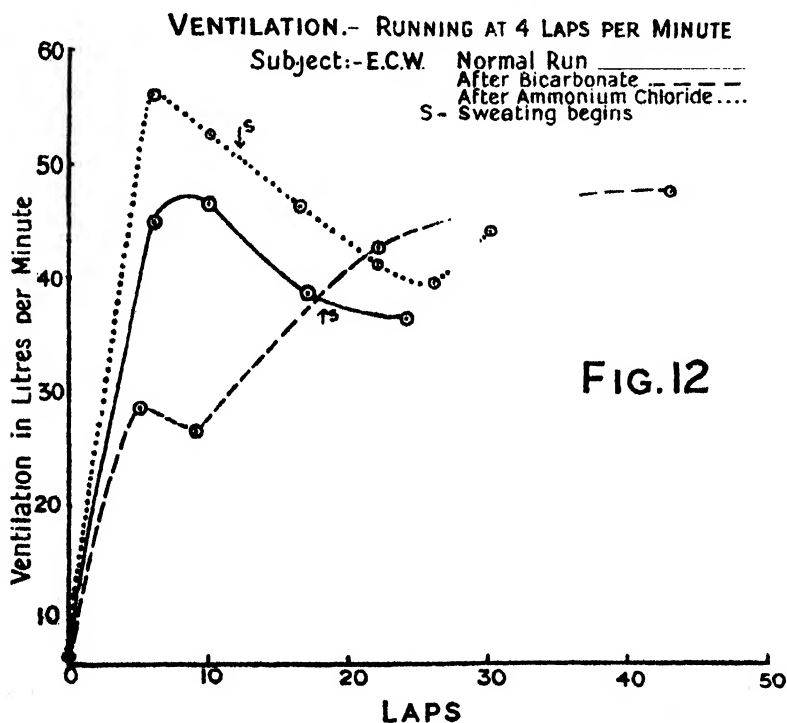


FIG. 12.—Pulmonary ventilation of E.C.W. running at rate of four laps per minute (7.28 m.p.h.) (a) Four hours after taking 560 c.c. of warm tea and 10 grms. of sodium bicarbonate; (b) two hours after taking 200 c.c. of warm tea and 10 grms. of ammonium chloride (some of which was vomited), and (c) after taking 560 c.c. of warm tea alone.

*The Temperature of the Body.*—The controversy over the question whether muscular work raises the internal temperature of a healthy man may be

\* 'Journ. Physiol.,' vol. 55, p. 265 (1921).



considered settled. Numerous observations\* have been published already, and it is unnecessary to give in detail further experiments. In fifty observations upon the temperature of the urine or rectum of four healthy young men, medical students, after muscular work of various degrees, a rise has been found in each case; the average increase was  $1.45^{\circ}$  F. ( $0.80^{\circ}$ ), and the maximum  $3.9^{\circ}$  ( $2.17^{\circ}$ ),  $102.9^{\circ}$  ( $39.39^{\circ}$ ) after a game of Rugby football; the temperature was over  $102^{\circ}$  ( $38.89^{\circ}$ ) in five cases, over  $101^{\circ}$  ( $38.33^{\circ}$ ) and under  $102^{\circ}$  ( $38.89^{\circ}$ ) in four cases and between  $100^{\circ}$  ( $37.78^{\circ}$ ) and  $101^{\circ}$  in twenty-four cases. This rise of temperature should be regarded as beneficial; oxidation occurs more rapidly when the internal temperature has been raised above  $98.6^{\circ}$  ( $37^{\circ}$ )†, the excitability of the respiratory centre is increased, the beat of the heart is quickened and the blood gives up its oxygen more readily to the tissues.‡

Discomfort or distress appeared to be more closely associated with a high temperature of the skin than with a high rectal temperature. This can be explained by the relationship between the circulation and temperature of the skin; dilatation of the cutaneous blood vessels makes an extra demand upon the heart in order to maintain the pressure of the blood. The exposure of a large surface of the skin during muscular work facilitates the evaporation of sweat, which prevents the temperature from exceeding the optimum and causes constriction of the cutaneous blood vessels.

*Results and Conclusions.*—In the dyspnoea produced by running there is a disturbance of the acid-base equilibrium of the body; the relief of second wind is the result of the various adjustments towards equilibrium. This accommodation is effected chiefly by the respiration, circulation and excretion by the kidneys and skin.

During dyspnoea there is a rise, on the onset of second wind a fall, in the percentage of carbon dioxide in the expired and alveolar air; the tension of oxygen in the alveoli shows smaller variations from the condition at rest.

The sense of discomfort during dyspnoea is associated with increased pulmonary ventilation, the sense of relief at the onset of second wind with diminished ventilation. The sensation of relief depends not upon the amount

\* Pembrey and Nicol, 'Journl. Physiol.,' vol. 23, p. 386 (1898). Pembrey, Arkle, Bolus and Lecky, 'Guy's Hospital Reports,' vol. 57, p. 283 (1902). In these papers the literature of the subject is considered. See also 'Committee on Physiological Effects of Food, Training and Clothing on the Soldier,' Second and Fourth Reports, 1908 ('Journ. R.A.M.C.,' vol. 12, p. 211 (1909); vol. 13, p. 592 (1909)).

† Sutton, 'Journ. Path. and Bact.,' vol. 13, p. 62 (1909).

‡ Barcroft and A. V. Hill, 'Journ. Physiol.,' vol. 39, p. 416 (1909-10).

of change alone, but the amount in relation to the starting value and the time in which the change occurs : it follows the law of excitation.

The average output of carbon dioxide during dyspnoea was 14.5 and during second wind 10.1 times the resting value ; the corresponding increases in the absorption of oxygen were 10.7 and 8.5.

Oliguria, or anuria, appears as a constant feature during running, even after the subject has taken 560 c.c. of tea as a diuretic. This oliguria leads to a temporary retention of acid, which assists the body to get rid of carbon dioxide and obtain oxygen ; the water spared is available for excretion by the lungs and skin, and will produce by evaporation a far greater cooling than it would if it were discharged as urinary water.

There was a conservation of water during the earlier stages of the run until sweating began ; this indicates that the oliguria is not secondary to the increased loss of water by the skin and lungs. Control experiments showed that the condition of the cutaneous blood vessels and the presence or absence of sweating were not the main causes of the renal inactivity. Oliguria was not observed at a rate of exercise below five miles per hour, even when the experimenter was in a hot room and was sweating profusely. The suspension of the activity of the kidneys during running appears to be due to an early outflow of constrictor impulses to the renal vessels. After strenuous exercise albumin appears in the urine of healthy subjects, apparently as a sign of the previous want of arterial blood in the kidneys ; the muscles and nervous system have a greater need of arterial blood.

The internal temperature of the body is raised by muscular work to 101° or 102° (38.33°, 38.89°) ; this is beneficial, for the oxidation in the tissues occurs more rapidly above 98.6 (37°), the excitability of the respiratory centre is increased, the beat of the heart is quickened and the blood gives up its oxygen more readily to the tissues, and its carbon dioxide to the alveolar air.

Discomfort or distress is associated with a high temperature of the skin ; the dilatation of the cutaneous vessels makes an extra demand upon the heart in order to maintain the pressure of the blood.

The adjustment of the acid-base equilibrium of the body can be disturbed on either side by the ingestion of sodium bicarbonate and ammonium chloride respectively.

Under ordinary conditions in a well-trained man doing vigorous muscular work dyspnoea must be regarded as a protective mechanism.

*The Titration of Amino- and Carboxyl-Groups in Amino-Acids, Polypeptides, etc. Parts I-III.—Investigations with Aqueous Solutions.*

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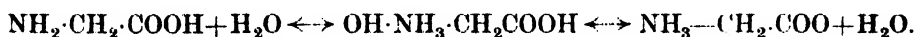
The development of the modern theory of titration and its application to analytical operations have greatly increased the scope of the volumetric method, and added considerably to its precision and refinement. In the following pages it will be shown on theoretical grounds that both the basic and acidic groups in amphoteric electrolytes are capable of estimation by a variety of acidimetric and alkalimetric methods, and experimental data will be presented to demonstrate that considerable accuracy is possible when the titrations are controlled by the conditions demanded by theoretical considerations.

These methods find a practical application in the determination and identification of amino-acids as well as of the more complex ampholytes which result from the hydrolytic breakdown of proteins; and results obtained by such methods are much more nearly quantitative than those resulting from actual separation and weighing of the constituents or their derivatives—a general method of attack in the past. Only two processes have hitherto been available for the estimation of amino-acids by titration, both depending on the acidity of the carboxyl group, that of Sørensen being carried out in presence of formol, and that of Foreman in presence of alcohol: these methods have remained to a large extent empirical and of somewhat limited application.

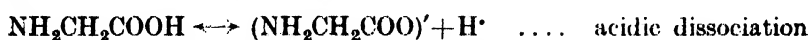
For clearness in presentation, the behaviour of amino-acids in aqueous solutions will be considered first. In the experimental investigations, the determinations in alcoholic and formaldehyde solutions recorded in the subsequent papers were the first to be carried out.

## PART I.—THE THEORY OF TITRATION APPLIED TO AMINO-ACIDS.

Glycine may be taken as an example of a typical amino-acid. In aqueous solution it can exist in several forms: unhydrated, with hydrated amino group, and as an internal salt formed by the mutual neutralisation of adjoining amino and carboxylic residues.



As well as these there are the ions formed in virtue of the ability of glycine to function as both acid and base.



By the law of mass-action, for a given temperature and solvent the three non-ionized molecules (*i.e.*, acid, hydrate and internal salt) are present in unchanging proportion irrespective of dilution, and may therefore for convenience be regarded as consisting of a single individual, namely, undissociated glycine (*cp.* Walker, 1905 (1) ).

Assuming that the acidic and basic dissociations are governed by the usual expressions,

$$K_a = \frac{[\text{H}^+] \times [\text{A}^-]}{[\text{HA}]}$$

$$K_b = \frac{[\text{B}^+] \times [\text{OH}^-]}{[\text{BOH}]}$$

we can regard the compound as if it were a mixture of an acid and a base, with ionization constants  $K_a$  and  $K_b$  respectively, each being independent of the other. The problem, therefore, is reduced to determining the conditions under which a base and an acid may be titrated in presence of one another; this is dependent upon the values of  $K_a$  and  $K_b$ . Further, when a dicarboxylic acid is to be estimated, we have to consider two values for  $K_a$ , *viz.*,  $K_{a1}$  and  $K_{a2}$ ; with a diamino acid the separate constants  $K_{b1}$  and  $K_{b2}$  must be taken into account; and with a mixture of ampholytes we have a series of values  $K_a, K_a', K_a'' \dots$  and  $K_b, K_b', K_b'' \dots$ . Amino-acids, polypeptides and the like are in general only weakly ionized so that  $K_a$  and  $K_b$  are small.\*

The titration of weak acids and weak bases in a mixture has been investigated

\* Bjerrum has recently put forward the theory that the generally accepted *dissociation constants* for amino-acids are not the "true" but "apparent" dissociation constants. Whichever view prove true, the conclusions reached below remain from a practical point of view unaffected.

by Noyes (2), Bjerrum (3) and others, and the conclusions may be briefly summarised as follows. In titrating an acid with a strong base the "end-point" is determined by the strength of the acid, *i.e.*, by the magnitude of  $K_a$ . The stronger an acid the less alkaline will be its titration end-point.\* Similarly, the weaker a base the more acid is its titration end-point. Provided the values  $K_a'$  and  $K_a''$  are of sufficiently different magnitude two acids present together may be titrated separately, the stronger first to a more acid end-point, then the weaker to a more alkaline end-point. Two bases in a mixture may be titrated similarly. For the successful titration of a stronger acid (base) in presence of a weaker one, it is necessary that throughout the range of  $P_H$  prevailing during the course of the titration the weaker acid (base) should remain unappreciably dissociated. The weaker acid (base) may then be titrated by taking to that higher (lower) value of  $P_H$  at which it in turn is completely dissociated. The assumption here is that the acid (or base) itself is scarcely ionized, but that the sodium salt or acid-hydrochloride is highly ionized. This has been proved by a number of workers to be true in the case of acids and bases of the "strength" that we are considering in dealing with amino-acids.

#### *Application to an Amino-acid.*

The possibility of separately titrating acids and bases in a mixture can be ascertained by a graphical method in which the percentage dissociation of each constituent is represented as a function of the hydrogen ion exponent. It is the object of the present section to utilise such methods in order to ascertain the conditions under which estimations of basic and acidic groups in amino-acids may be carried out. Fig. 1 shows the dissociation curves for glycine. Ordinates represent percentage dissociation, abscissæ the corresponding  $P_H$ .

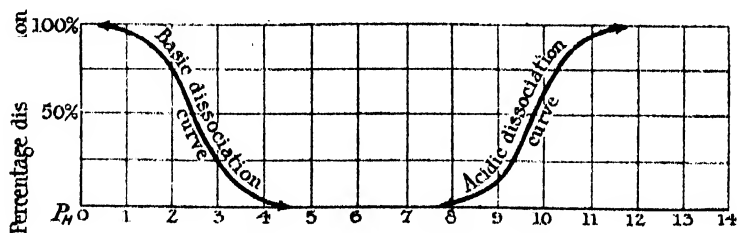


FIG. 1.—Dissociation Curves of Glycine.

\* Thus the addition of one equivalent of soda (1) to an equivalent of hydrochloric acid produces a neutral solution, (2) to an equivalent of acetic acid an alkaline solution, (3) to an equivalent of boric acid (a very weak acid) a highly alkaline solution.

The curves are constructed from the formulæ,

$$\log \frac{1}{[H^+]} = \log \frac{1}{K_a} + \log \frac{\alpha}{(1-\alpha)},$$

for the acidic dissociation curve;

$$\log \frac{1}{[H^+]} = \log \frac{K_b}{K_w} + \log \frac{(1-\alpha)}{\alpha},$$

for the basic dissociation curve.

The values for  $K_a$  and  $K_b$  being those determined by Winkelblech (4) from conductivity measurements. (The derivation of these formulæ and an elementary treatment of dissociation curves can be found in Clark's "The Determination of Hydrogen Ions," 1920 (5).)

Reference to the diagram will show that glycine dissociates as an acid only appreciably when the solution is more alkaline than  $P_H = 7.75$ . It is almost entirely (99 per cent.) ionized at  $P_H 11.75$  and still more completely in still more alkaline reactions. Further, above  $P_H 4.43$  glycine practically ceases to function as a base. It may be assumed, therefore, from theoretical considerations that the addition of one equivalent of alkali to one equivalent of glycine solution will give a virtual end-point at  $P_H = 11.75$ . In alkaline solution, then, glycine will be functioning as a monobasic acid, the basic character of the  $NH_2$  group being "submerged." The practical end-point for the titration of the carboxyl is  $P_H = 11.75$ , fixed by the value of the acidic dissociation constant  $K_a$ . It is likewise deduced that in acid solutions the acidic dissociation of glycine is inappreciable; at  $P_H 7.75$  it falls to 1 per cent. It may be assumed therefore that the amino group may be titrated without interference from the opposing acidic tendency of the carboxyl group, which fails to ionize to any appreciable extent owing to the acid reaction. At  $P_H 0.43$  the dissociation curve approaches very close to the 100 per cent. dissociation abscissa, and this is therefore fixed as the end-point to which the titration must be carried. The same process of deduction may be applied to the titration of the other amino-acids.

In general, it can be shown that a stronger acid,  $K_{a1}$  (or, base,  $K_{b1}$ ), may be titrated in presence of a weaker one,  $K_{a2}$  ( $K_{b2}$ ), without appreciable error when

$$\log \frac{1}{K_{a2}} \cong 4 + \log \frac{1}{K_{a1}}, \text{ for acids,}$$

$$\log \frac{K_{b1}}{K_w} \cong 4 + \frac{K_{b2}}{K_w}, \text{ for bases.}$$

In the limiting cases taken in these formulæ there will be no appreciable overlapping of the several dissociation curves; at the determinant  $P_H$  at which the "stronger" constituent is 99 per cent. dissociated, there will be only 1 per cent. dissociation of the "weaker." The error due to non-dissociation of the stronger constituent is therefore only 1 per cent. Since the ionization ratio,  $\alpha/(1-\alpha)$ , of weak acids and bases increases tenfold with unit change of  $P_H$ , this error is reduced to 0.1 per cent. when the fig. 5 is substituted for fig. 4 in the above equations.

Acids and bases may be titrated separately in presence of one another, without serious error or distortion of titration curves, when

$$\log \frac{1}{K_a} \cong 4 + \log \frac{K_b}{K_w}.$$

In this limiting case the basic and acidic dissociation curves overlap at a point representing 1 per cent. dissociation. Therefore, at that  $P_H$  where the acid constituent is 99 per cent. dissociated (*i.e.*, neutralised) the basic constituent is ionized to the extent of only 0.0001 per cent., and *vice versa*.

(A) *Monoamino-Monocarboxylic Acids.*

In Table I are given the ionization constants of various monoamino-mono-carboxylic acids, together with the calculated titration end-points.

Table I.

	$K_a$	Carboxyl titration end-point (99 per cent. dissn.).	$K_b$	Amino titration end- point (99 per cent. dissn.).
		$P_H$		$P_H$
1. $\alpha$ -Alanine	$1.9 \times 10^{-10}$	11.73	$5.1 \times 10^{-12}$	0.71
2. Glycine	$1.8 \times 10^{-10}$	11.75	$2.7 \times 10^{-12}$	0.43
3. Leucine	$1.8 \times 10^{-10}$	11.75	$2.3 \times 10^{-12}$	0.37
4. Phenyl-alanine	$2.5 \times 10^{-9}$	10.60	$1.3 \times 10^{-12}$	0.10
5. Valine	<i>ca.</i> $2 \times 10^{-10}$	<i>ca.</i> 11.7	$2.0 \times 10^{-12}$	0.3

$K_a$  and  $K_b$  for alanine, glycine and leucine were determined by Winkelblech (4) from conductivity measurements, for phenyl-alanine by Kanitz (6). The values for valine are due to the present writer.

Fig. 2 shows the dissociation curves for these acids (except valine).

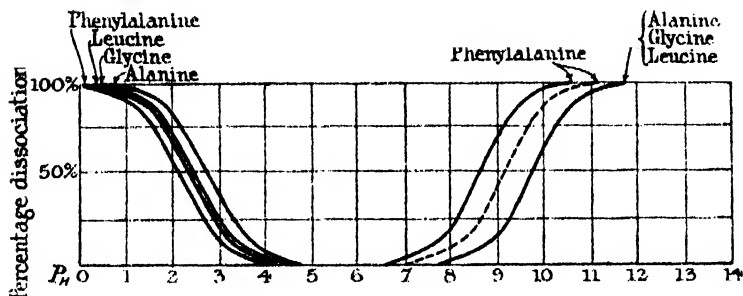


FIG. 2.—Dissociation Curves of "Neutral" Amino-Acids.

### Estimation of $NH_2$ .

It will be seen that at  $P_H$  0.7 to 0.1 the amino dissociation becomes almost complete. If  $P_H$  0.4 to 0.5 were fixed as the titration end-point the error due to unneutralised residue should be comparatively small, even in the case of phenyl-alanine, which has the smallest  $K_b$  value and therefore the most acid end-point. At  $P_H$  0.4, 100 equivalents of phenyl-alanine would require 98 equivalents of HCl and at  $P_H$  0.5, 97.5; the error being 2 and  $2\frac{1}{2}$  per cent. respectively.\*

It may be concluded therefore that the amino groupings in a mixture of monoamino-monocarboxylic acids may be estimated by titration with alkali to  $P_H$  0.5 or, better, 0.4

A considerable correction for a blank will be necessary, because an appreciable amount of hydrochloric acid must be added to water containing *no amino-acids* to reduce the  $P_H$  to 0.5.

An alternative method proposed is to titrate to some definite  $P_H$  value corresponding with an intermediate point on the titration curve, a fixed fraction of the amino-acid present being neutralised at a given  $P_H$ . For example, at the  $P_H$  value numerically equal to  $\log K_b/K_w$  the amino group is exactly half neutralised; since

$$\log \frac{K_b}{K_w} = \log \frac{1}{H}$$

at the half titration point. In each case the  $P_H$  for the mid titration point is obtained by adding 2.00 to the values in the last column of Table II.

The advantages of this procedure are that the titration is taken to a point which can readily be reached with N/10 HCl and that the "blank" correction to be applied is insignificant. The disadvantages are that there is less change

\* Or even less, see below



in the direction of the ideal titration curve at the 50 per cent. neutralisation point than there is in the neighbourhood of the full titration point; that owing to the buffering effect and the consequent flatness of the titration curve the addition of successive (corrected) amounts of the titrant has relatively less effect on the  $P_H$ ; and that when dealing with a mixture of amino-acids the mid titration  $P_H$  values are not quite coincident.

#### *Estimation of Carboxyl.*

It is seen from the chart that at  $P_H$  11.75 the carboxyl in glycine is for practical purposes completely ionized as sodium salt, the remaining acids of the group\* at a slightly less alkaline figure. To estimate carboxyl therefore in the "neutral" acids it would appear necessary to fix the end-point at a  $P_H$  not less alkaline than 11.75. At this reaction the presence of  $NH_2$  in the molecule will have no measurable effect on the titration.

#### *In Presence of Strong Acids and Bases.*

The method is capable of application to hydrolysis mixtures containing the "neutral" amino-acids together with a strong mineral acid such as sulphuric or a base such as NaOH. The strong acid or alkali is first neutralised by bringing the mixture to  $P_H$  5.5, for which purpose methyl-red may be used as an indicator. A neutral mineral salt is thus produced which remains dissociated at all the  $P_H$  ranges employed, and therefore does not prevent the subsequent estimation of amino and carboxyl by the same method as that indicated above. At  $P_H$  5.5 none of the monoamino-monocarboxylic acids are appreciably ionized, and hence the titration of the mineral acid or strong base will not be interfered with. If, however, the formation of the neutral salt involve the production of an ion which reacts with the amino-acid the normal dissociation is disturbed (as shown by Michaelis, 1920 (7)), and the method is no longer applicable.

#### *Strength of Solutions; Temperature, etc.*

$K_a$  and  $K_b$  for glycine, alanine and leucine as determined by Winkelblech (4) apply to dilutions of from 32 to 1024. It is unlikely that concentrations of these amino-acids would be met with in practice that would fail to give true titration results owing to variation of  $K_a$  or  $K_b$  with dilution.

The titrations are best carried out at 25°, the temperature at which  $K_a$  and  $K_b$  (Table I) were determined. At 17.5° and 18°  $K_a$  and  $K_b$  (as

\* Tyrosin is discussed with the dicarboxylic acids and histidine with the diamino acids or reasons which will appear below.

determined by Michaelis and Rona (8) and by Dernby (9) ) have slightly lower values than those given in Table I. Hence at room temperature, titration end-points would be slightly further removed from true neutrality than at 25°.

The dissociation constants for phenyl-alanine were determined by Kanitz. The dilution was not stated and the magnitude of the constants seems less well established than in the case of the other acids. The acid titration curve (see experimental section below) points to a value for  $K_a$  slightly lower than that indicated by Kanitz, in which case  $K_a$  for phenyl-alanine is not quite so widely divergent from  $K_a$  for glycine, alanine and leucine (determined by Winkelblech). The dotted line in the dissociation curve is that deduced from the titration. It is possible also that  $K_b$  for phenyl-alanine more nearly approximates to  $K_b$  for the acids determined by Winkelblech, in which case the very slightly lower readings predicted for  $NH_2$  in phenyl-alanine on titrating to  $P_H$  0.5 would not in practice be met with. It is hoped to report on this at a later date.

#### *Applicability.*

The above treatment includes all the neutral ampholytes resulting from protein hydrolysis except cystine (a diamino-dicarboxylic acid) and tryptophane. Colorimetric observations with indicators, recorded in Part III, show that the dissociation constants for tryptophane are nearly identical with those for alanine. In the case of cystine also it is safe to assume that the dissociation constants are of the same order as those of the other acids of the series (*cp.* titration values given in subsequent sections), and the method then becomes of general applicability to all the monoamino-monocarboxylic acids of biochemical importance (and to cystine).

A *hydroxy-monoamino-monocarboxylic acid*, viz., tyrosine, behaves on titration in alkaline solution as a dibasic acid and is therefore treated in the next section dealing with acid ampholytes. The behaviour of serine (another *hydroxy-monoamino-monocarboxylic acid*) remains to be investigated.

The imino group in histidine (unlike that in tryptophane) functions as a weak base, and this amino-acid is therefore discussed with the basic ampholytes.

#### (B) *Dicarboxylic Acids ; Acid Ampholytes.*

The monoamino-dicarboxylic acids (viz. glutamic and aspartic acids) contain *one* carboxyl group which is strongly ionized in neutral solution. In the case of *tyrosine* it is found experimentally that the phenolic radical titrates as a

monobasic acid in alkaline solution, and hence tyrosine is to be regarded as a dibasic acid.

Below are the end-points calculated from the values of  $K_a$  and  $K_b$  as in the case of the neutral ampholytes.

Table II.

	$K_a$	Carboxyl titration end- points (99 per cent. dissn.).		Amino titration end- points (99 per cent. dissn.).
		$P_H$		$P_H$
Glutamic acid.	$\left\{ \begin{array}{l} K_1 \ 4.1 \times 10^{-5} \\ K_{a2} \ 1.6 \times 10^{-10} \end{array} \right\}$	$\left\{ \begin{array}{l} 6.39 \\ 11.80 \end{array} \right\}$		0.18
Aspartic acid	$\left\{ \begin{array}{l} K_{a1} \ 1.5 \times 10^{-4} \\ K_{a2} \ 1.4 \times 10^{-10} \end{array} \right\}$	$\left\{ \begin{array}{l} 5.83 \\ 11.85 \end{array} \right\}$	$1.2 \times 10^{-12}$	0.08
Tyrosine	$\left\{ \begin{array}{l} K_{a1} \ 4 \times 10^{-10} \\ K_{a2} \ 4 \times 10^{-11} \end{array} \right\}$	$\left\{ \begin{array}{l} 11.4 \\ 12.4 \end{array} \right\}$	$2.6 \times 10^{-12}$	0.42

All values refer to 25° C.

The constants for the first and the second acidic dissociation of tyrosine (the latter due to phenolic OH) are derived from the titration curve\* (see below). That for the second acidic dissociation of aspartic acid was deduced by Hopfield, Halstead, Brennan and Acree from H-electrode measurements of buffer solutions used for culture media in an *unpublished* paper read to the American Chemical Society ('Proc.,' Chicago (Sept. 6-10, 1920)), a summary of which is given in 'Science,' 1920 (10). The remaining values may be found in Landolt-Börnstein (11) and in Michaelis (12); those for glutamic acid are due to Holmberg (13) (diazoacetic ester method, and hydrolysis), and for aspartic acid to Winkelblech (5) and Lunden (conductivity).

In fig. 3 are shown the dissociation curves deduced from these constants. The curves for tyrosine, deduced from the titration, are represented by dotted lines.

The first dissociation of aspartic and glutamic acids approaches completion at  $P_H$  6.4. Hence, it is concluded that one carboxyl group in these acids

\* Kanitz gives the values  $K_a = 4 \times 10^{-9}$  and  $K_{a2} = 4 \times 10^{-10}$  (from conductivity and hydrolysis respectively) but the evidence of the titration curve points consistently to values  $K_{a1} = 4 \times 10^{-10}$  and  $K_{a2} = 4 \times 10^{-11}$ .

may be estimated by titrating with N/10 soda to this hydrogen ion exponent. The same procedure may be adopted in dealing with a mixture of dicarboxylic

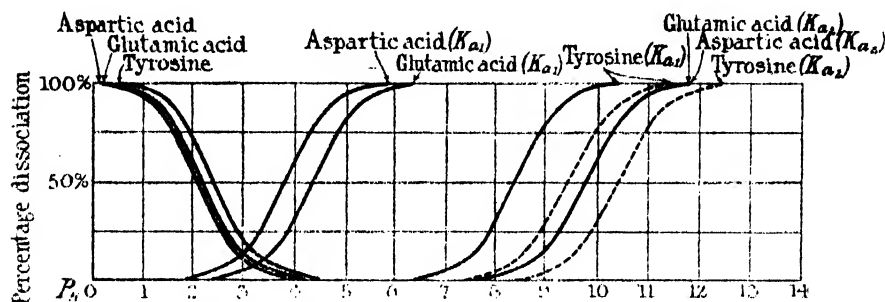


FIG. 3. — Dissociation Curves of "Acidic" Ampholytes.

acids, tyrosine, and the acids of the "neutral" group, because the  $NH_2$ 's and the remaining carboxyls are inappreciably dissociated at the stated  $P_H$ .

The total amino and carboxyl present may be estimated (according to theory) by titrating to strongly acid and alkaline end-points respectively as described before, the same corrections being made for the "blank."  $P_H = 12.4$  is high enough for the estimation of tyrosine as a dibasic acid and gives an ample margin with the remaining acids.

If the solution contain a strong mineral acid or alkali it may first be brought to  $P_H 6.4$ ; whereby the strong electrolyte is titrated practically to completion together with the first carboxyl of aspartic and glutamic acids. The remaining carboxyl (plus tyrosine—OH) may then be estimated by continuing the titration to the alkaline end-point, and the  $NH_2$  by continuing the titration to the acid end-point.

The constants for the recently discovered *hydroxy-glutamic acid* have not yet been determined.

### (C) Basic Ampholytes.

The dissociation constants of the basic amino-acids are known with less certainty than those of the two groups discussed above.

The following values are given by Kanitz (14) :—

Histidine.	Arginine.	Lysine.
$K_a$ $2.2 \times 10^{-9}$	$K_a > 1.11 \times 10^{-14}$	$K_a$ about $1.2 \times 10^{-12}$
$K_{b1}$ $5.7 \times 10^{-9}$	$K_{b1} < 1.0 \times 10^{-7}$	$K_{b1} < 1.0 \times 10^{-7}$
$K_{b2}$ $5 \times 10^{-13}$	$K_{b2}$ $2.2 \times 10^{-12}$	$K_{b2}$ $1.1 \times 10^{-12}$

These figures are reproduced in this form in several tables of physical constants, but it would appear that no less than four of the six values given for arginine

and lysine are misprinted. For  $K_{b1}$  arginine and  $K_{b1}$  lysine, the sign  $<$  should be replaced by  $>$ ; for  $K_a$  arginine,  $>$  should be replaced by  $<$ , and  $K_a$  lysine should read "about  $1.2 \times 10^{-11}$ , not " $1.2 \times 10^{-12}$ ." Only by these alterations are values obtained which are in concordance with experimental observation of "titration" curves and of the neutrality of the mono-acid salts of lysine and arginine. Further, the alterations indicated are required by the context and by the values deduced in Kanitz's own paper.

Speaking of arginine mononitrate and lysine monohydrochloride, he says (*loc. cit.*, p. 492)---"Was besagen würde, dass die zuletzt genannten Salze nicht messbar hydrolysiert sind. Dieses Ergebnis wird dadurch bestätigt, dass in Gegenwart von M/10 Lösungen der erwähnten Salzgemische das Methylacetat, selbst nach Monaten, nicht nennenswert gespalten ist. Die erste Basedissoziationskonstante des Arginins und Lysins ist also mindestens  $1 \times 10^{-7}$ , und wäre somit aus der Leitfähigkeit der freien 'Basen' mit einiger Genauigkeit bestimmbar, wenn dieselben kohlensäurefrei herstellbar wären."

It follows that  $K_{b1}$  for arginine and lysine is equal to or greater than  $1 \times 10^{-7}$

$$\text{i.e., } K_{b1} \geq 1 \times 10^{-7}.$$

With regard to the acidic dissociation constant of arginine he says (*loc. cit.*):---"Die Leitfähigkeit des Argininnatriums ergab sich so bei  $v = 32$  annähernd gleich der Leitfähigkeit der Natronlauge und zeigte für weitere Verdünnungen die für die letztere charakteristische Leitfähigkeitsabnahme. Das Arginin besitzt demzufolge keine Säureeigenschaft." It may be concluded therefore that the acidic dissociation constant is *less* than that of water (*i.e.*,  $K_a < 1 \times 10^{-14}$ ), and *not* greater, as is implied by the value given by Kanitz, *viz.*,  $> 1.11 \times 10^{-14}$ .

The following is the only reference in the text of the paper to the acid dissociation constant of lysine:---"Die Leitfähigkeit des Lysinnatriums ergab, dass dasselbe selbst bei  $v = 1024$  über 50 pro cent. hydrolysiert ist. Die Säuredissoziationskonstante des Lysins beträgt somit *ca.*  $1.2 \times 10^{-11}$ ." When the various values are tabulated, however, "*ca.*  $1.2 \times 10^{-12}$ " is substituted for the above! The former value is in fairly good agreement with titration curves, as shown below.

In Table III are given the amended constants with the calculated titration end-points (99 per cent. ionization):---

Table III.

	$K_a$	$\log 1/K_a$	end-point.	$K_b$	$\log K_b/K_{H^+}$	end-point.
			$P_H$			$P_H$
Histidine	$2.2 \times 10^{-9}$	8.65	10.65	$\begin{cases} (1) 5.7 \times 10^{-9} \\ (2) 5 \times 10^{-12} \end{cases}$	$\begin{cases} 5.76 \\ 1.7 \end{cases}$	$\begin{cases} 3.76 \\ -0.3 \end{cases}$
Arginine	$< 1.1 \times 10^{-14}$	$> 13.95$	$> 15.95$	$\begin{cases} (1) > 1.0 \times 10^{-7} \\ (2) 2.2 \times 10^{-12} \end{cases}$	$\begin{cases} > 7.0 \\ 2.35 \end{cases}$	$\begin{cases} > 5.0 \\ 0.35 \end{cases}$
Lysine	$1.2 \times 10^{-11}$	11-10.7	13-12.7	$\begin{cases} (1) > 1 \times 10^{-7} \\ (2) 1.1 \times 10^{-12} \end{cases}$	$\begin{cases} > 7.0 \\ 2.05 \end{cases}$	$\begin{cases} > 5.0 \\ 0.05 \end{cases}$

Temperature 25° C.

From these values of  $K_a$  and  $K_b$  are plotted the dissociation curves, fig. 4, calculated by the usual formulæ. The dotted curve is the first basic dissociation of lysine obtained from experimental titration curve values.

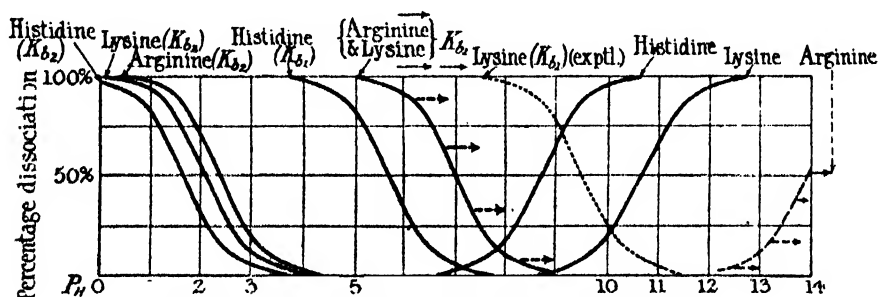


FIG. 4.—Dissociation Curves of "Basic" Ampholytes.

The following deductions can be made from the curves. At  $P_H$  about 7.0, one  $NH_2$  in both arginine and lysine functions basically. Histidine is only very slightly ionized with respect to one basic group, and the other groups, basic and acidic, are inappreciably ionized. Arginine and lysine in solution may, therefore, be estimated by titrating one amino group to  $P_H 7$ , for which purpose phenol-red can be employed as indicator. Strong mineral acids or alkalis present in the solution are estimated together with the stronger  $NH_2$  in this titration. Total basic groups (plus free alkali or minus free acid) are estimated by titrating to a very acid end-point, the usual blank correction being made. Total carboxyl (plus strong acid or minus free alkali) is estimated by titrating to a very alkaline end-point, e.g.,  $P_H$  13.5 to 14. In the latter

titration arginine will fail to show any titratable  $\text{CO}_2\text{H}$  or only a very small fraction of the percentage calculated from its formula. In the titration of basic groups  $=\text{NH}$  in histidine will tend to give a low reading unless the titration is carried to a very acid  $\text{P}_\text{H}$  region. A solution of histidine may be estimated by titrating the  $\text{NH}_2$  (which is rather more strongly basic than in the "neutral" acids) to an end-point of  $\text{P}_\text{H}$  3.8. (Very accurate results may be so obtained using brom-phenol-blue as indicator. Details will be found in Part III, together with some remarks on the controverted "basicity" of histidine.)

*Proline and Oxypoline*, which contain  $=\text{NH}$  but no  $-\text{NH}_2$ , are not dealt with in this paper, which is more especially concerned with amino-bodies.

#### (D) Polypeptides.

The dissociation constants of the three dipeptides alanyl-glycine, glycyl-glycine and leucyl-glycine have been determined by Euler, and are almost identical for each of the three bodies. Both acid and basic constants are distinctly higher than in the case of the monoamino-monocarboxylic acids.

In Table IV are tabulated Euler's values (15) for  $K_\text{a}$  and  $K_\text{b}$ , and the titration end-points calculated therefrom.

Table IV.

	$K_\text{a}$	$\log 1/K_\text{a}$	end-point (99 per cent. dissn.).	$K_\text{b}$	$\log 1/K_\text{b}$	end-point (99 per cent. dissn.).
			$\text{P}_\text{H}$			$\text{P}_\text{H}$
Alanyl-glycine	$1.8 \times 10^{-8}$ ....	7.75	9.75	$2 \times 10^{-11}$	10.7	1.3
Glycyl-glycine	$1.8 \times 10^{-8}$ ....	7.75	9.75	$2 \times 10^{-11}$	10.7	1.3
Leucyl-glycine	$1.5 \times 10^{-8}$ ....	7.83	9.83	$3 \times 10^{-11}$	10.5	1.5

Fig. 5 gives the corresponding dissociation curves. From these it will be seen that the dipeptides in question function as acids at  $\text{P}_\text{H}$  above 5.75 to 5.84, and as bases below  $\text{P}_\text{H}$  5.3 to 5.5, and there is no appreciable overlapping of the acidic and basic dissociations. There is practically complete (99 per cent.) dissociation as an acid at  $\text{P}_\text{H}$  9.75 to 9.84, and as a base at  $\text{P}_\text{H}$  1.3 to 1.5, and these hydrogen ion exponents are therefore the required end-points in the respective titrations of acidic and basic groups.

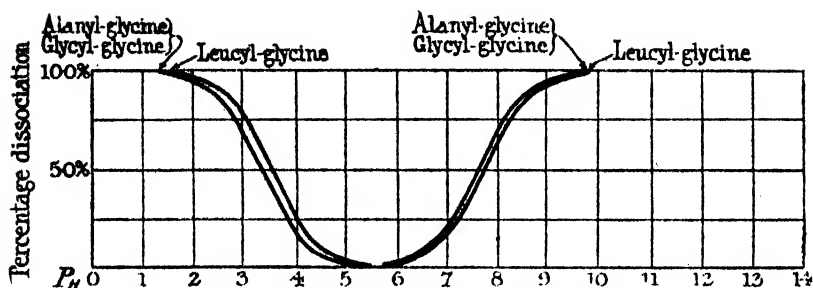


FIG. 5.—Dissociation Curves of Dipeptides.

(E) *General procedure for mixtures of Amino-acids and Polypeptides.*

It is now possible to deduce appropriate titration end-points for the estimation of carboxyl and amino groups in mixtures of the three classes of amino-acids and of dipeptides of the type considered above. For this purpose the four dissociation diagrams may be regarded as superimposed one on another, and the extent of ionization of the separate groups considered in the manner already described.

By titrating to  $P_H 7$  the following will be estimated :—One half the carboxyl-groups of glutamic and aspartic acid, one half the amino-groups of lysine and arginine, together with any mineral acid or caustic alkali present in the mixture ; the appropriate signs, +ve or -ve, being applied to acidic or basic constituents. All the radicals which are thus estimated are practically completely dissociated in the form of salts at this hydrogen ion concentration, while the remaining groups, carboxyl and amino, are inappreciably ionized (less than 1 per cent., except in the case of histidine, tyrosine and perhaps polypeptides (carboxylic), where the dissociation is slightly greater).

Total carboxyl and phenolic (tyrosine) groups, plus mineral acid if present, will be estimated by titration to the alkaline end-point ( $P_H = 13$ , being the maximum alkalinity necessary). Amino-groups, having no measurable basicity at such a "reaction," will not enter into the titration. Arginine will behave as though it contains no carboxyl group. The "blank" correction, mentioned above, must be applied.

Total amino-groups, and the imino-group in histidine (but not in tryptophane), plus caustic alkali if present, will be estimated by titration to highly acid end-point. Here again a "blank" correction will be necessary.

In dealing with a mixture containing excess of some particular amino-acids special regard must be paid to their separate dissociation curves. For example, to estimate free hydrochloric acid present in small quantities in a concentrated



mixture of alanyl-glycine, glycyl-glycine and leucyl-glycine, the titration should be ended at  $P_H6$  instead of  $P_H7$ , because the polypeptides will be ionized to a less extent at  $P_H6$  than at  $P_H7$ . Although the *percentage* ionization of the polypeptide (as an acid) is small at  $P_H7$ , if the substance is present in sufficient amount there will be an appreciable error, since the ionized fraction will take part in the titration in addition to the hydrochloric acid.

In problems of this kind, in which certain constituents are known to be present to excess, the most satisfactory end-points may be determined by constructing, in place of the ordinary dissociation-curves, diagrams in which *ionic-concentrations* (instead of *percentage* ionization) are plotted against  $P_H$ , as described by Bjerrum (see Prideaux, "The Theory and Use of Indicators," (16)).

It will be clear also that there is sufficient divergence between the values  $K_a$  or  $K_b$ ,  $14 - pK_{a1}$ , and  $pK_{a1}$ , of the "neutral," "basic" and "acidic" ampholytes respectively, to enable at any rate each of these three groups to be determined separately when present simultaneously in a mixture. For this purpose the given mixture is carried from highly acid to highly alkaline reaction by successive small additions of HCl and NaOH; the various portions of the resulting complete titration curve can be resolved and identified in a manner to be explained hereafter.

## PART II.—EXPERIMENTAL VERIFICATION— $P_H$ MEASUREMENTS, AMINO-ACID ESTIMATIONS.

### (A) *Estimation of $NH_2$ .*

No reference can be found in the literature to any previous attempt to estimate  $NH_2$ -groups in amino-acids and the like by titration with acid. Nevertheless,  $P_H$  readings of buffer-solutions of glycine with varying amounts of HCl have been made with great accuracy by Sørensen (17); and Eckweiler, Noyes and Falk (18) have more recently examined the  $P_H$  values of several other amino-acids and dipeptides in the presence of acid.\* The results obtained by these workers may be expressed in the form of titration curves, provided certain corrections are introduced. (1) In the first place the "blank-correction" mentioned in Part I must be applied. Suppose a mixture of  $a$  c.c. of N/10 amino-acid with  $x$  c.c. of N/10 HCl has a given  $P_H$ , determined by hydrogen electrode measurement. Now a certain quantity of N/10 HCl will have to be added to a blank consisting of *water alone* (containing no amino-acid) in

\* Most of these values have been reproduced and confirmed by the author.

order to produce the same final volume of solution  $(a+x)^*$  having the given  $P_H$ . Let the volume of N/10 HCl required to reduce the water alone to the given  $P_H$  be  $w$ ; then the amount required to reduce the amino-acid alone to the given  $P_H$  will be  $x-w$ , which is the *corrected* titration figure. (2) In the second place Sørensen's determinations of  $P_H$  were carried out on solutions of the same total volume in each case, but containing varying amounts of both amino-acid and HCl. They can be expressed in the form of titration readings if they are re-calculated to show the effect on  $P_H$  of adding *increasing* amounts of HCl to a constant amount of amino-acid solution.

(1)  $P_H$  of N/10 HCl in water alone.—To avoid the necessity of calculating the blank correction in each case, a curve has been constructed showing the volume of N/10 HCl in 100 cc. of HCl-aq. solutions of varying  $P_H$ . From this, the correction for a *given* total volume of titrated fluid may easily be obtained. In constructing the curve, the degree of dissociation ( $\alpha$ ) of HCl was taken throughout at 0.9. This value gives the corrections with a sufficient degree of accuracy, since they become significant as  $P_H$  approaches 1 where  $\alpha$  approaches 0.9. The various values of  $P_H$  in Table V were calculated direct from the formula :—

$$-\log [H^+] = -\log \alpha [HCl]$$

Table V.

Cc. of N/10 HCl in 100 c.c. of solution.	Concentration of Acid.	$P_H$ .
0	0.000	7.00 ( $t = 23^\circ$ )
2	0.002	2.75
10	0.010	2.05
25	0.025	1.65
50	0.050	1.35
75	0.075	1.17
100	0.100	1.05

These figures are plotted in the correction curve†, fig. 6, which agrees very closely with experimental determinations by the hydrogen electrode.

#### Titration of $NH_2$ in Glycine.

In Table VI, columns *a* and *b*, are given the composition of mixtures of glycine with hydrochloric acid in varying proportions having the  $P_H$  values given in column *f*, as experimentally determined by Sørensen (17). In columns

\* Within the experimental accuracy.

† The scale for the correction curve is quite distinct from the scale for the glycine titration curve in the same figure.

$c$  and  $d$  the same relative proportions are given but corresponding with a fixed amount of glycine, i.e., in the form of titration readings. In column  $g$  is entered the blank correction read from the correction curve *per* 100 c.c. of solution for each value of  $P_H$  in column  $f$ . In column  $h$  are given the corrections for the given total volume of titrated fluid (column  $e$ ). Column  $i$  contains the corrected titration volume (i.e., c.c. of N/10 HCl added in order to reduce the amino-acid alone to the given  $P_H$ ). Thus, values in column  $d$  = c.c. of N/10 HCl required to titrate amino-acid plus water to given  $P_H$  (uncorrected reading); values in column  $h$  = c.c. of N/10 HCl required to titrate water alone to given  $P_H$  (blank correction for the given final volume); values in column  $i$  = c.c. of N/10 HCl required to titrate amino-acid alone to given  $P_H$  (corrected reading).

Table VI.

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
Sørensen's figures		Equivalent to		Total volume of liquid after titration ( $e = c + d$ ).	Corresponding $P_H$ values.	Blank for 100 c.c. total volume (read from curve).	Blank for given total volume, $e$ . ( $h = ge/100$ ).	Corrected volume of N/10 HCl used in titration ( $i = d - h$ ).
N/10 glycine.	N/10 HCl.	N/10 glycine.	N/10 HCl used in titration.					
c.c.	c.c.	c.c.	c.c.	c.c.		c.c.	c.c.	c.c.
9.5	0.5	5	0.263	5.263	3.679	—	—	0.263
9	1	5	0.55	5.55	3.341	—	—	0.55
8	2	5	1.25	6.25	2.922	1	0.06	1.19
7	3	5	2.143	7.143	2.607	3	0.21	1.93
6	4	5	3.3	8.3	2.279	5	0.4	2.93
5	5	5	5.0	10	1.932	13	1.3	3.7
4	6	5	7.5	12.5	1.645	25	3.1	4.4
3	7	5	11.6	16.6	1.419	42	7.0	4.66
2	8	5	20	25	1.251	60.5	15.1	4.9
1	9	5	45	50	1.146	80	40	5.0

The corrected and uncorrected titration readings are plotted against  $P_H$  in fig. 6. The corrected curve shows a remarkable agreement with that calculated theoretically from the basic dissociation constant. The theoretical curve is calculated from the well-known approximation formula derived from the mass law

$$\log \frac{1}{[H^+]} = \log \frac{K_b}{K_a} + \frac{[\text{base}]}{[\text{salt}]} \quad (1)$$

in which  $[base]$  is simply taken as the concentration of glycine remaining unneutralised by the titrant (i.e., excess concentration of glycine over HCl

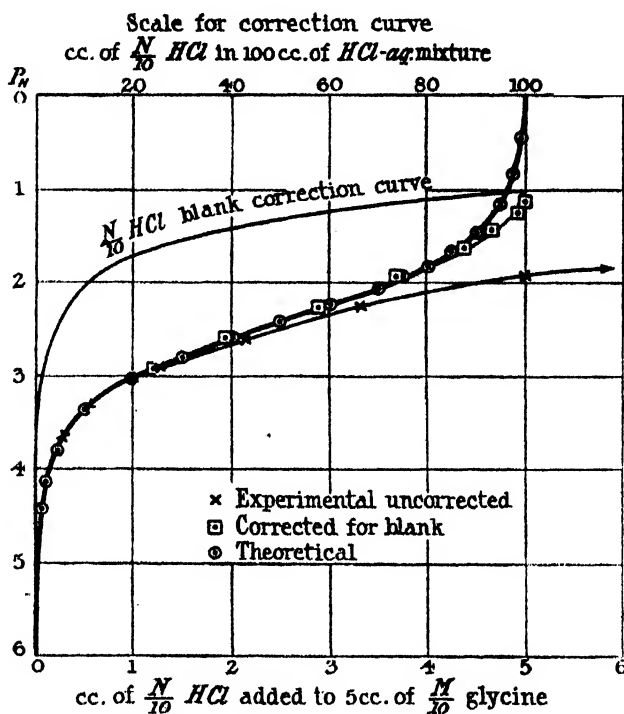


FIG. 6.—Estimation of  $-NH_2$  in glycine.

added, at each stage in the titration), and  $[salt]$  as the glycine hydrochloride produced (which is equivalent to the concentration of hydrochloric acid added at each stage in the titration).  $K_b$  is taken as  $2.7 \times 10^{-12}$ , the value determined by Winkelblech (see Table I above). Values of  $P_H$  calculated theoretically in this manner after the addition of various amounts of HCl to 100 equivalents of glycine are given in Table VII.

Table VII.—Theoretical Deduction of Titration Curve of Glycine+HCl (Corrected).

Equivalents of HCl added to 100 equivs. of glycine.	Ratio [base]/[salt]	log ratio.	log $K_a/K_{a'}$	$P_H = \log 1/H^+$
2	49	+1.69	2.43	4.12
4	24	+1.38	2.43	3.81
10	9	+0.95	2.43	3.38
20	4	+0.60	2.43	3.03
30	2.33	+0.37	2.43	2.80
40	1.5	+0.18	2.43	2.61
50	1.0	0.00	2.43	2.43
60	0.667	-0.18	2.43	2.25
70	0.429	-0.37	2.43	2.06
75	0.333	-0.48	2.43	1.95
80	0.25	-0.60	2.43	1.83
85	0.176	-0.75	2.43	1.68
90	0.111	-0.95	2.43	1.48
95	0.053	-1.28	2.43	1.15
97.5	0.0256	-1.59	2.43	0.84
99.0	0.0101	-1.996	2.43	0.434

*Calculation of Actual (Uncorrected) Titration Readings from the Corrected or Theoretical Curves.*

The process that has just been employed for correcting observed titration values for the amount of acid necessary to bring the solvent alone to a given  $P_H$  is expressed algebraically by the following formula :—

$$i = d - ge/100 \dots\dots\dots (2)$$

where

$i$  = corrected amount of acid used in titration to a given  $P_H$ .

$d$  = Uncorrected amount of acid used in titration to a given  $P_H$ .

$g$  = Blank for 100 c.c. (No. of c.c. of acid necessary to bring solvent alone to the given  $P_H$  and to a final volume of 100 c.c.).

$e$  = Volume of titrated fluid after addition of  $d$  c.c. of acid.

The blank correction,  $g$ , may be read from the curve which is based on the formula

$$P_H = -\log \cdot \alpha [HCl]$$

which may be re-written

$$P_H = -\log (\alpha \cdot g/100 N) \dots\dots\dots (3)$$

where

$g$  = No. of c.c. of HCl

$N$  = normality of HCl.

By the use of formulæ (2) and (3) it is possible to deduce the uncorrected (experimental) curve from the theoretical curve. (A) For example, suppose one wishes to determine the proportion in which glycine and HCl must be mixed in order to produce a solution of a given  $P_H$ . From the mass-law equation (1), p. 456 above, or from the theoretical curve deduced therefrom, one obtains figures which are inaccurate in so far that no allowance is made for the effect of the added HCl on the solvent. More HCl will have to be added than that indicated by the formula, because it is necessary to add HCl to bring the solvent also to the same  $P_H$  value. This correction is introduced by the application of the formula

$$d = \frac{e + g/100}{1 - g/100}, \dots\dots\dots (4)$$

which has been obtained by transposing formula (2) after the substitution of  $(c+d)$  for  $e$ , where  $c$  = volume of glycine (or other amino-acid) solution taken for titration, the remaining symbols having the same meaning as before.

Formula (1), then, enables one to calculate the amount of HCl necessary to bring the amino-acid alone to the given  $P_H$  (viz., the value  $e$ ); formula (4) corrects this by adding the amount of HCl necessary to bring the solvent itself to the same  $P_H$  (giving the value  $d$ ).

(B) In a similar way one can calculate the  $P_H$  (corrected for effect of solvent) of a mixture of amino-acid with HCl in known concentrations, by making use of formulæ (1), (3) and (4).

*Titration with N/1 HCl in place of N/10 HCl.*—The titration of basic groups in amino-acids is better accomplished with N/1 HCl than N/10 for two reasons. Firstly, the most acid reaction attainable by titration with N/10 HCl\* is  $P_H$  about 1.1 which is considerably less acid than the basic end-point (99 per cent. neutralisation) of glycine and most other amino-acids; while by the use of N/1 HCl the end-point and still more acid reactions may be reached. Secondly, the use of a weaker titrant causes greater dilution of the solution and a corresponding increase in the correction factor (blank for the solvent), which is directly proportional to the final volume of the titrated fluid. For example, the titration of 5 c.c. of N/10 glycine solution produces with an equivalent of, (1), N/10 HCl a final volume of 10 c.c., (2), with N/1 HCl a final volume of 5.5 c.c.: the *equivalents* of HCl for the blank in the two cases are in the ratio 10 to 5.5 respectively.

*Blank Correction for N/1 HCl.*—As with N/10 HCl a curve has been

\*  $P_H$  for N/10 HCl itself being 1.05.

constructed to show the volumes of N/1 HCl which must be added to water alone to produce 100 c.c. of solutions of given  $P_H$  values. The blank correction for a given final volume may be calculated from this curve by simple proportion.

The correction curve is derived from the formula

$$P_H = \log \overline{[H^+]} \quad \log \alpha [HCl].$$

Values of  $\alpha$  are calculated from conductivity measurements, due to Kohlrausch. Since these were determined at 18° it is not strictly accurate to apply the blank corrections to titrations carried out at 25° C., or to theoretical titration curves calculated from values of  $K_a$  determined at 25° C.

Table VIII.

C.c. of N/1 HCl in 100 c.c. of solution.	[HCl].	$\alpha$ (18°)	[H <sup>+</sup> ].	$P_H$
100	1.0	0.785	0.785	0.11
50	0.5	0.853	0.4265	0.37
30	0.3	0.875	0.2625	0.58
20	0.2	0.891	0.1782	0.75
10	0.1	0.914	0.0914	1.04
5	0.05	0.938	0.0469	1.33
3	0.03	0.949	0.02847	1.55
2	0.02	0.957	0.01914	1.72
1	0.01	0.964	0.00964	2.02
0.5	0.005	0.973	0.00487	2.31

The experimental (uncorrected) curves for titration with N/10 HCl and N/1 HCl respectively may be deduced from the theoretical (corrected) curve—calculated from formula (1)—by the application of formulæ (3) and (4).

(The variation in strength of HCl (N/1 or N/10) denotes a variation in the magnitude of the symbol N in formula (3), which implies a variation in  $g$ , for a given value of  $P_H$ . The latter symbol and  $c$ , which depends upon it, both occur in the formula (4) for calculating the experimental curve, the form of which accordingly depends on the strength of the titrant employed.)

In fig. 7 the uncorrected titration curve of glycine with N/1 HCl is given. This may be compared with that with N/10 HCl, fig. 6. In Table IX the method of calculating the corrected curve, according to formula (2), is shown.

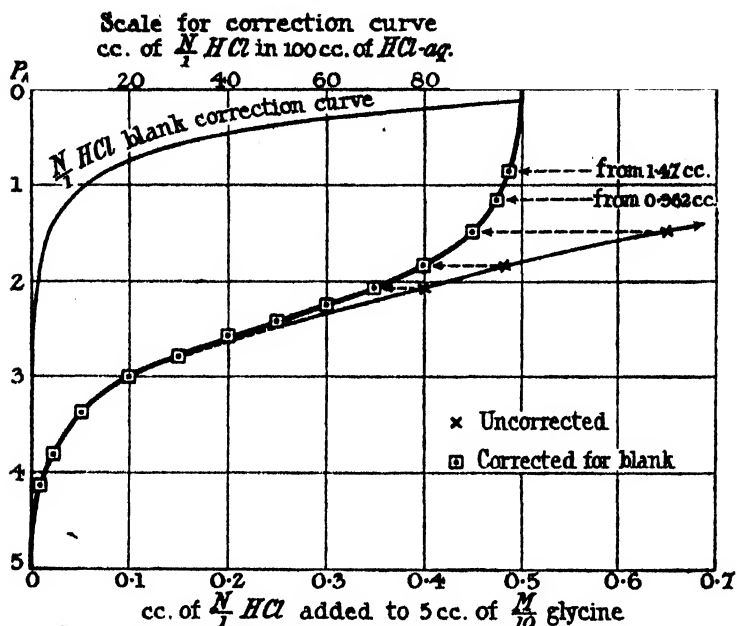


FIG. 7.—Titration of 5 c.c. M/10 glycine with NHCl.

 Table IX.—Titration of  $\text{NH}_2$  in Glycine with N/1 HCl. (Calculated.)

(c)	(d)	(e)	(f)	(g)	(h)	(i)
N/10 glycine.	N/1 HCl used in titration.	Total volume of liquid after titration.	Corresponding $P_H$ values.	Blank for 100 c.c., total volume (read from curve).	Blank given total volume %.	Corrected of volume N/1 HCl used in titration.
		$(e = c + d)$			$h = ge/100$	$i = d - h$
c.c.	c.c.	c.c.		c.c.	c.c.	c.c.
5.0	0.40	5.4	2.06	1	0.05	0.35
5.0	0.48	5.48	1.83	1.5	0.08	0.40
5.0	0.65	5.65	1.48	3.5	0.20	0.45
5.0	0.96	5.96	1.15	8	0.48	0.48
5.0	1.47	6.47	0.85	15	0.98	0.49
5.0	(4.65)	(9.65)	(0.43)	(43)	4.15	0.50

In the final portion of the titration the blank correction assumes comparatively large magnitudes, hence slight error in the determination of  $P_H$  or in estimating the "blank for 100 c.c." may lead to a serious error in the corrected titration figure. The final portion of the titration curve will therefore tend to be



represented by a series of points distributed on either side of a line asymptotic to the vertical, which line itself is the form of the ideal (or theoretical) curve.

#### *Presence of Sodium Chloride.*

Sørensen's determinations referred to above were carried out in presence of 0.1 N sodium chloride. Accurate results may be obtained equally in the absence of a neutral salt, as is shown by the work of Tague (*vide infra*) who titrated amino-acids with soda\* in absence of salt, and of Sørensen (19) who compared the  $P_H$  of ampholyte solutions with and without salt. Care is required to ensure absence of carbon dioxide, more especially when titrating acidic groups with alkali.\*

#### *Extrapolation of Uncompleted Curves.*

The corrected titration curves of any single basic (or acidic) groups are identical in form, but their position on the  $P_H$  axis varies according to the magnitude of  $K_b$  (or  $K_a$ ). Hence it is possible when only the early part of a titration curve has been determined (that part where the blank corrections can, in general, be ignored) to continue it by extrapolation to the end-point. It is, therefore, possible quite accurately to estimate substances whose end-points calculated from the constant  $K_b$  are even more acid than the solution used for the titration. The geometrical mid-point of the curve represents the  $P_H$  resulting when one-half equivalent of the titrant has been added to one equivalent of the substance. This  $P_H$  value is numerically equal to  $\log K_b/K_w$ † for a base, and  $\log 1/K_a$ † for an acid.

#### *Deduction of $K_b$ for some Amino-acids and Dipeptides from Titration Curves.*

Just as it is possible to foretell the titration curve of a body when  $K_b$  is known, so, conversely,  $K_b$  can be deduced when the titration curve has been obtained. Below,  $K_b$  for a number of amino-acid and dipeptides are calculated from titrations. In cases where  $K_b$  was already known (conductivity measurements, etc.) the new values are generally in excellent agreement, in several cases the dissociation constants were before unknown and are here given for the first time.

Eckweiler, Noyes and Falk (*loc. cit.*) have determined the  $P_H$  of mixtures of simple ampholytes with varying amounts of HCl (and NaOH), "as a necessary preliminary to a more satisfactory understanding of the more complex

\* Titration of carboxyl groups, see below.

† Denoted by  $14 - pK_b$  and  $pK_a$  respectively.

bodies" (proteins). They were chiefly concerned with a discussion of the isoelectric point, and do not deal with the estimation of amino-acids or the dependence of the titration curves upon the value of  $K_b$ , nor is the blank correction for solvent introduced into their results. With glycine their results are in good agreement with those of Sørensen which have been utilised above, and with those of Tague, and of the author.

Fig. 8 shows the central portion of the basic titration curves of three amino-acids and three dipeptides. The crosses represent experimental

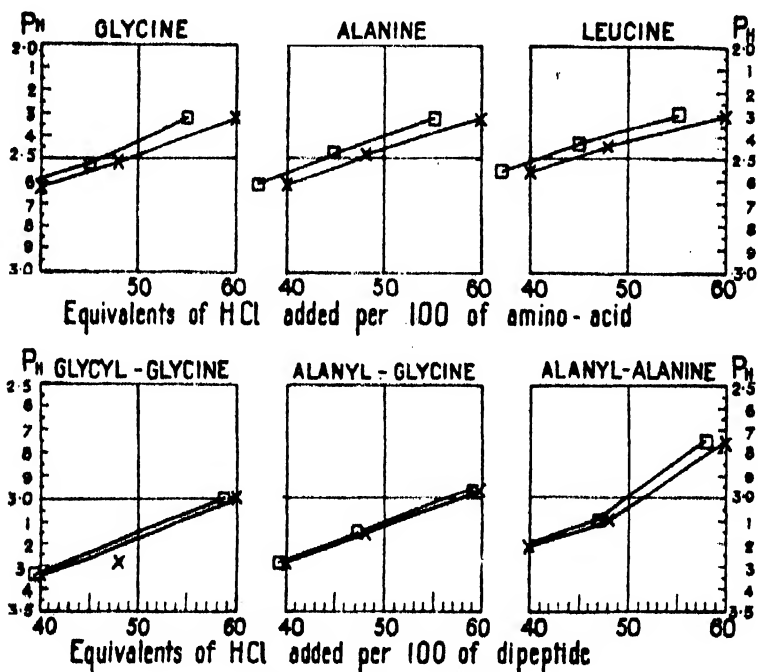


FIG. 8.—Calculation of  $K_b$  from titration curves.

readings, the squares the readings as corrected for the amount of acid required to bring the "solution without solute" to the same hydrogen ion concentrations. The mid-points of such corrected curves are tabulated below together with the values for  $K_b$  calculated therefrom, and the previously determined values are also added for comparison.

Table X.—Deduction of  $K_b$  from Titration Curves.

	Titration mid-point (corrected).	$K_b$ on assumption that $P_H$ at mid-point $= 14 - \log 1/K_b$ .	$K_b$ previous values, from conductivity, etc.
Glycine ....	$P_H$ 2.41	$2.6 \times 10^{-12}$	$2.7 \times 10^{-12}$ (Winkel- blech)
Alanine ....	2.40	$2.5 \times 10^{-12}$	$\left\{ \begin{array}{l} 3.4 \times 10^{-12} \\ 5.1 \times 10^{-12} \end{array} \right\}$ ..
Valine ....	2.3	$2 \times 10^{-12}$	— ..
Leucine ....	2.36	$2.3 \times 10^{-12}$	$2.3 \times 10^{-12}$ ..
Glycyl-glycine ....	3.15	$1.4 \times 10^{-11}$	$2 \times 10^{-11}$ (Euler)
Alanyl-glycine ....	3.1	$1.3 \times 10^{-11}$	$2 \times 10^{-11}$ ..
Alanyl-alanine ....	3.0	$1 \times 10^{-11}$	—
Lysine $K_{b1}$ ....	(9.5)*	$(3.2 \times 10^{-5})$	$> 1 \times 10^{-7}$ (Kanitz)
$K_{b2}$ ....	2.0*	$1 \times 10^{-12}$	$1.1 \times 10^{-12}$ ..

\* See p. 474 *et seq.*

The complete titration curves for alanine, valine and leucine are very similar to that shown for glycine and correspond very closely with the form of the theoretical curve. Fig. 9 shows: (1) the results obtained by titrating 25 c.c. of N/10 alanyl-glycine solution with N/10 HCl and diluting to 50 c.c.

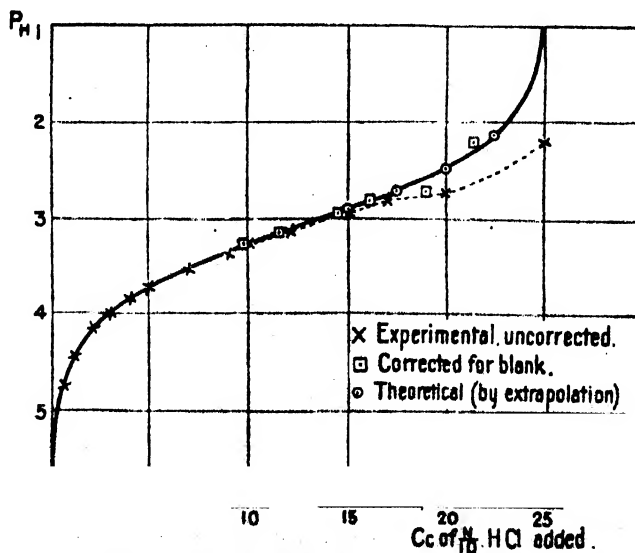


FIG. 9.—Titration of alanyl-glycine. Final vol. made up to 50 c.c. in each case (Eckweiler).

with water before taking the  $P_H$  (Eckweiler, Noyes and Falk); (2) the corrected titration curve; and (3) the theoretical curve obtained by extrapolating from the mid-titration point  $P_H$  3.1. (The lower part of the curve, where no correction is necessary, corresponds closely with a basic dissociation of  $14 - pK_b = 3.1$ .)

Table XI.—Correction of Alanlyl-glycine Titration.

Volume of 0.1 N HCl added to 25 c.c. of 0.1 N alanlyl-glycine.	Resulting $P_H$ on making solution up to 50 c.c.	Correction for 100 c.c. (from curve).	Correction for 50 c.c.	Corrected volume of 0.1 N HCl for 25 c.c. of 0.1 N alanlyl-glycine.
c.c.				c.c.
0.5	4.74			0.5
1	4.42			1
2	4.19			2
3	3.99			3
4	3.84			4
5	3.71			5
7	3.53			7
9	3.39			9
10	3.28	0.5	0.25	9.75
12	3.15	1	0.5	11.5
15	2.97	1	0.5	14.5
17	2.83	1.7	0.9	16.1
20	2.74	2	1.0	19.0
25	2.21	7	3.5	21.5

Table XII.—Algebraic Extrapolation of Titration Curve.

(Titration of 25 c.c. of N/10 alanlyl-glycine.)

c.c. of N/10 HCl added.	log-base/salt.	$P_H$ ( $= 14 - pK_b + \log\text{-base/salt}$ ).
12.5	0	3.1
15	-0.18	2.92
17.5	-0.37	2.73
20	-0.60	2.5
22.5	-0.95	2.15
24.75	-2.00	1.1

Very similar results have been obtained with other dipeptides.

#### *Effect of Dilution on $P_H$*

The theoretical (or the corrected) titration curve shows the  $P_H$  of mixtures of varying proportions of amino-acid, etc., with HCl; it is identical for any dilution of the solution, provided there is no change in the value of the dissociation constant. The blank correction, on the contrary, is directly

proportional to the volume of the solution. The effect of increasingly diluting a solution containing a given ratio *amino-acid* : *HCl* will therefore have the effect of increasing the divergence between the corrected and uncorrected curve. In the earlier parts of the titration the blank correction is negligible and therefore the dilution here inappreciably affects the  $P_H$ . In the later stages of the titration, however, where the ratio *HCl* : *amino-acid* is greater, the blank correction becomes appreciable, and then the greater the dilution the greater is the amount of *HCl* that must be added to a given amount of ampholyte in solution to produce a given  $P_H$ .

#### *Use of Quinhydrone Electrode.*

The use of the hydrogen electrode for determining  $P_H$  values finds its chief drawback in the complexity and difficulty of the technique, but the process becomes comparatively simple with the substitution of the quinhydrone for the hydrogen electrode.\* The present writer has found that the quinhydrone electrode furnishes a ready method of estimating acids and bases in pigmented solutions where indicators would be inapplicable, and of obtaining titration curves with much greater ease and rapidity than is possible with a hydrogen electrode. He has been able to utilise the quinhydrone electrode for the estimation of bases of any strength, and for any but the weakest acids ( $K_a < 10^{-6}$ ), provided no reaction occurs with the quinhydrone.

Dealing with *amino-acids*, determinations such as the following have been made: Determination of an amino-acid by titrating the  $NH_2$  group; determination of total amino groups present in a mixture; determination of lysine or histidine, alone or in a mixture of "neutral" amino-acids; determination of glutamic acid, alone or in a mixture of "neutral" amino-acids (by titration of the stronger carboxyl group).

Two types of procedure have been employed:--(1) The complete corrected or *ideal titration curve* has been deduced, or, (2) the potentiometer was set at a given reading and titrant added until zero deflection was attained, the corrected volume of titrant so required being proportional to the original concentration of titratable body.

The order of accuracy obtained was equal to that attaching to the volumetric apparatus employed. Fuller details and experimental values are given elsewhere ('J. Chem. Soc.').

\* I am indebted to Mr. V. La Mer for this suggestion.

## (B) Determination of COOH.

Tague (20) has proposed to estimate amino-acids by titration with N/10 soda. Using the hydrogen-electrode he obtained titration curves for the following five acids: phenylalanine, tyrosine, lysine dihydrochloride, and glutamic acid. He also corrected the results for the effect of adding NaOH to a blank consisting of water alone.

Although Tague's results show different end-points for different amino-acids no explanation was offered of their dependence on the value of  $K_a$  and no attempt was made to fix the end-points by calculation. Indeed, although it could be shown that the titration curves for the above amino-acids are in close conformity with those demanded by acids having the respective  $K_a$  values, Tague supposes that the dissociation constant  $K_b$  continually decreases with increasing addition of soda. This assumption was made in order to reconcile the observed stoichiometric end-point with the formula of Walker

$$a^2 = \frac{K + K_a u}{1 + K_b u},$$

(where  $a$  is the concentration of the hydrogen ion,  $K$  the ionisation constant of the water,  $K_a$  the ionisation constant of the ampholyte as an acid,  $K_b$  the constant of the ampholyte as a base, and  $u$  the concentration of the undissociated molecules).

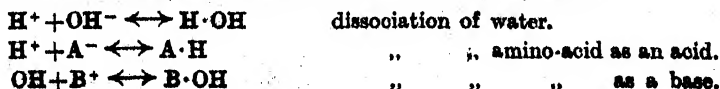
Since, however, it has been shown above that glycine and other amino-acids behave as a mixture of a weak base (with a definite dissociation "constant"  $K_b$ ) with a weak acid (with a definite "constant"  $K_a$ ) and since the values of  $K_a$  and  $K_b$  are such that appreciable ionization as an acid occurs only in alkaline solution and as a base only in acid solution, it would appear impossible as well as unnecessary to assume that the constants vary with  $P_H$ .

*Distinction between the Basic and Acidic "Dissociation-Residues" of an Ampholyte.*

The formula of Walker can be made applicable if two values of  $u$  are taken ( $u_b$  and  $u_a$ ), representing the undissociated molecules of the ampholyte considered as a base and as an acid respectively. The amended formula takes the form

$$a^2 = \frac{K + K_a u_a}{1 + (K_b/K) u_b}.*$$

\* Deduced in the same way as Walker's formula, from the equations



The assumption is here made that that form of the amino-acid which ionizes as a base is different from that form which ionizes as an acid. (Such a distinction is suggested in the equations at the beginning of this paper.)

It has already been proved by Loeb that this is the case with complex molecules such as gelatin—that the positive ampholyte is fundamentally different from the same ampholyte as a negative ion; from which it follows that the non-ionized molecule (dissociation residue) is different in the two cases.

### *Titration of Carboxyl in Glycine.*

In Table XIII the titration curve of glycine with N/10 soda is calculated from the formula

$$\log \frac{1}{[H^+]} = \log \frac{1}{K_a} + \log \frac{[A^-]}{[HA]}.$$

In fig. 10 the values thus obtained are plotted for comparison along with the experimental values of Tague. The agreement between the theoretical and experimental readings is remarkably close.

The relative molecular concentrations being

$H^+$	$OH^-$	$A^-$	$B^+$	$B \cdot OH$	$A \cdot H$
$a$	$b$	$c$	$d$	$u_b$	$u_a$
				basic dissociation residue.	acidic dissociation residue.

we have  $ab = K$  .....(5)

$ac = K_a u_a$  .....(6)

$bd = K_b u_b$  .....(7)

combining (7) and (5)  $d = K_b/K \cdot u_b \cdot a$  .....(9)

„ (6) and (5)  $c = K_a/K \cdot u_a \cdot b$  .....(10)

but,

$$a + d = b + c$$

and substituting from (9) and (10) for values  $c$  and  $d$ ,

$$a \{1 + (K_b/K) \cdot u_b\} = b \{1 + (K_a/K) \cdot u_a\} \text{ .....(12)}$$

multiplying by  $a$ , and substituting for  $b$  from (5)

$$a^2 = \frac{K + K_a \cdot u_a}{1 + (K_b/K) \cdot u_b}.$$

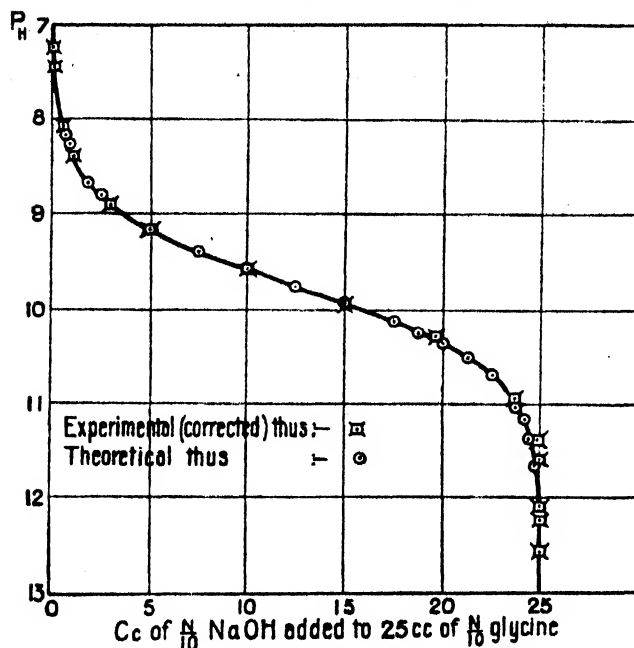

 FIG. 10.—Estimation of  $\text{—CO}_2\text{H}$  in glycine.

Table XIII.—Titration of 25 c.c. of N/10 Glycine.

$$K_a = 1.8 \times 10^{-10} \therefore \log 1/K_a = 9.75.$$

N/10 NaOH.	$[\text{A}^-]/[\text{HA}] = \text{salt/acid.}$	$\log \text{salt/acid.}$	$\log 1/\text{H} = P_H$ calculated.
c.c.			
0.5	0.02	-1.69	8.06
0.625	0.026	-1.59	8.16
0.75	0.031	-1.51	8.24
1.0	0.042	-1.38	8.37
1.25	0.053	-1.28	8.47
1.875	0.081	-1.09	8.60
2.5	0.111	-0.95	8.80
5.0	0.250	-0.61	9.15
7.5	0.429	-0.37	9.38
10	0.667	-0.18	9.57
12.5	1.000	0.00	9.75
15	1.500	+0.18	9.93
17.5	2.33	+0.37	10.12
18.75	3.00	+0.48	10.23
20	4.00	+0.60	10.35
21.25	5.67	+0.75	10.50
22.5	9.0	+0.95	10.70
23.75	19.0	+1.28	11.03
24.0625	25.67	+1.41	11.16
24.375	39.00	+1.59	11.34
24.6875	79.0	+1.90	11.65



*Deduction of  $K_a$  from Experimental Titration Curves.*

The following table gives the value of  $K_a$  deduced from experimental titration curves by the relation

$$P_H = -\log K_a \text{ at mid point,}$$

and it will be seen that these values are generally in good agreement with those obtained from conductivity measurements, hydrolysis, etc.

Table XIV.

	1		2
	From titration curves.		From conductivity, etc.
	$p \cdot K_a$	$K_a$	$K_a$
No.			
1. Glycine ....	9.75	$1.8 \times 10^{-10}$	$1.8 \times 10^{-10}$ (Winkelblech)
2. Alanine ....	9.75	$1.8 \times 10^{-10}$	$1.9 \times 10^{-10}$
3. Leucine ....	9.6	$2.5 \times 10^{-10}$	$1.8 \times 10^{-10}$ "
4. Phenylalanine ....	9.13	$0.75 \times 10^{-9}$	$2.5 \times 10^{-9}$ (Kanitz)
5. Glycyl-glycine ....	8.28	$0.53 \times 10^{-8}$	$1.8 \times 10^{-8}$ (Euler)
6. Alanyl-glycine ....	8.18	$0.66 \times 10^{-8}$	$1.8 \times 10^{-8}$ "
7. Alanyl-alanine ....	8.18	$0.66 \times 10^{-8}$	
8. Glutamic-acid { $K_{a1}$ ....	4.2	$6.3 \times 10^{-5}$	$6.3 \times 10^{-5}$ (Holmberg)
{ $K_{a2}$ ....	9.8	$1.6 \times 10^{-10}$	
9. Tyrosine . .... { $K_{a1}$ ....	9.4	$4 \times 10^{-10}$	$4 \times 10^{-9}$ (Kanitz)
{ $K_{a2}$ ....	10.4	$4 \times 10^{-11}$	
10. Lysine ....	10.7	$2 \times 10^{-11}$	$1.2 \times 10^{-11}$ "

The  $P_H$  values in Column 1 are based on  $P_H$  measurements by Sørensen, by Eckweiler, Noyes and Falk, by Tague, and by the present writer; the constants in Column III are taken from Landolt-Börnstein, p. 1185. Nos. 1 to 7 have been taken direct from the simple titration curves figs. 10, 11, 12. In the case of glutamic acid two curves are apparent, one succeeding the other without appreciable interference (owing to the wide difference in the two  $K_a$  values). In consequence each mid-point can be read independently without reference to the other curve. In the case of tyrosine, however, the values  $K_{a1}$  and  $K_{a2}$  are close enough to cause overlapping of the two dissociation curves and a single continuous curve results, which accordingly must be resolved into its two components in the manner described below before the mid points of each can be determined. In the case of lysine there is overlapping of the  $K_a$  curve and the  $K_{s1}$  curve.

*Dipeptides Behave as Monobasic Acids and Monoacidic Bases.*

It has been observed in the past that a greater quantity of acid or alkali must be employed when a dipeptide is titrated to a given  $P_H$  than when an

amino-acid is titrated to the same  $P_H$ . The explanation has been advanced that in the case of the dipeptide, addition of acid or alkali takes place at the enolised peptide linkage as well as at the terminal amino or carboxyl groups. Eckweiler, Noyes and Falk (*loc. cit.*, p. 302), who plotted amounts of acid added as ordinates against  $P_H$  as abscissæ, drew the following conclusions :—

“As compared with the amino-acids there was a very much smaller range where little or no buffer action was shown by the dipeptides. Aside from this the curves for the two series were practically parallel, the vertical differences between the two sets of curves show the difference in the amounts of acid and alkali required to bring the substances to the same  $P_H$  values. Much more acid or alkali was required for the dipeptides than for the amino-acids, the difference being due evidently to the  $-\text{CO}-\text{NH}-$  group of the dipeptides. The differences increase with increasing quantities of acid or alkali added, reach a maximum and then decrease again. The chemical nature of the  $-\text{CO}-\text{NH}-$  group readily accounts for these properties, acid combining with the  $-\text{CO}-\text{NH}-$  group alkali bringing about enol-lactim rearrangement and accompanying reaction.”

The view that the difference between the amounts of acid (or alkali) required to bring a dipeptide and an amino-acid respectively to the same  $P_H$  is a measure of the amount of acid (or alkali) taken up by the peptide linkage of the dipeptide would appear untenable when the titration-curves are viewed from the standpoint of the theory of titration. The titration curves for alanyl-glycine, alanyl-alanine and glycyl-glycine show a definite end-point corresponding to the addition of a molecular weight of acid or alkali to each molecular weight of the dipeptides. Further, the form of the curves is identical with that demanded for the neutralisation of a simple base or acid having the dissociation constants  $K_a$  and  $K_b$  of the given dipeptide. The fact that more acid or alkali is required for a polypeptide than for an amino-acid in titrating to a given  $P_H$  is due simply to the higher values of  $K_a$  and  $K_b$  for the dipeptide. The carboxyl group in the dipeptide being more strongly acidic than the same group in an amino-acid is neutralised at a less alkaline reaction, and the titration curve throughout is situated at a higher level, fig. 11; the amino group in the dipeptide being more strongly basic than the same group in an amino-acid is neutralised at a less acid reaction, and the titration curve is situated throughout at a lower level.\*

\* Since the titration curve corresponds to the addition of a *single* equivalent of acid or alkali, it seems legitimate to assume that the reaction occurs at the terminal  $-\text{NH}_2$  or  $-\text{CO}_2\text{H}$ , as is the case with amino-acids (which show perfectly analogous titration curves), and not at the peptide linkage.

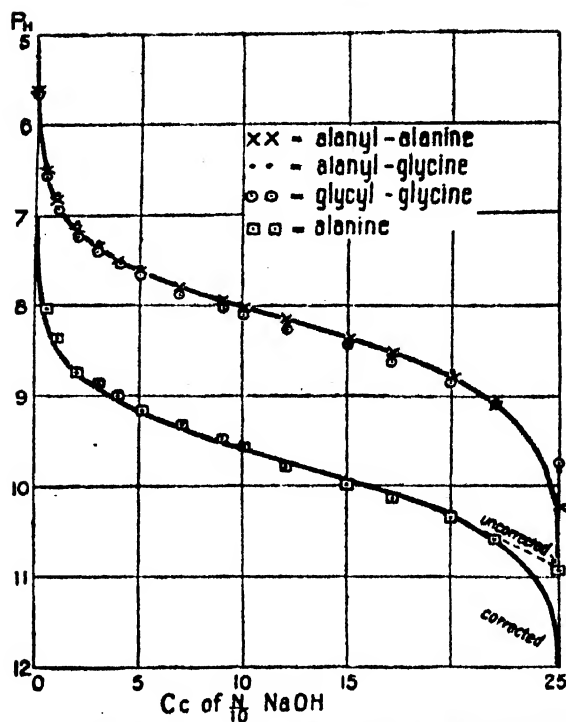


FIG. 11.—Titration of Alanyl-alanine, Alanyl-glycine, Glycyl-glycine, Alanine.

Scale for amino-acid titration. Cc of  $\frac{1}{10}$  NaOH added to 25 cc. of  $\frac{1}{10}$  amino-acid

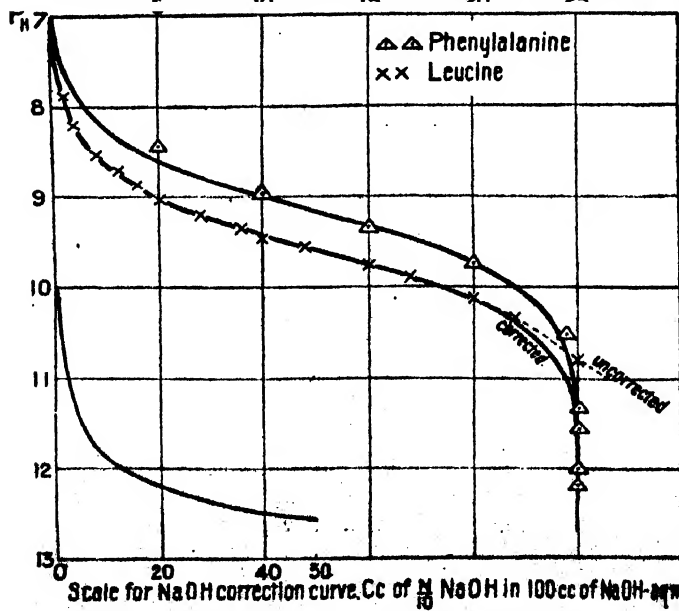


FIG. 12.—Titration of Carboxyl in Phenylalanine and Leucine.

It is possible that if the titrations were taken to very strongly acid and alkaline end-points there might be evidence of some additional combination with acid or alkali.

Readings are to be desired also with tri- and polypeptides in order to obtain information as to their basicity. The question is of some importance, because it has been shown by many observers that certain complex ampholytes such as proteins and protein derivatives possess acid and alkali binding power greater than can be accounted for by the number of free amino and carboxyl groups, and such binding power has been attributed to the peptide linkage.

### Compound Titration Curves.

In cases where there are several acidic or basic groups and there is overlapping of the several dissociation-curves, it has been found possible to deduce the effect on the resulting titration curve by a process of summation. The combined effect at each  $P_H$  value is determined by adding together the amounts of each group which would be neutralised at that  $P_H$  if it were present alone and independent of the others; these are the amounts shown in the single titration curves deduced from the respective dissociation constants.

Reference to the diagram for tyrosine (fig. 13) should make the method

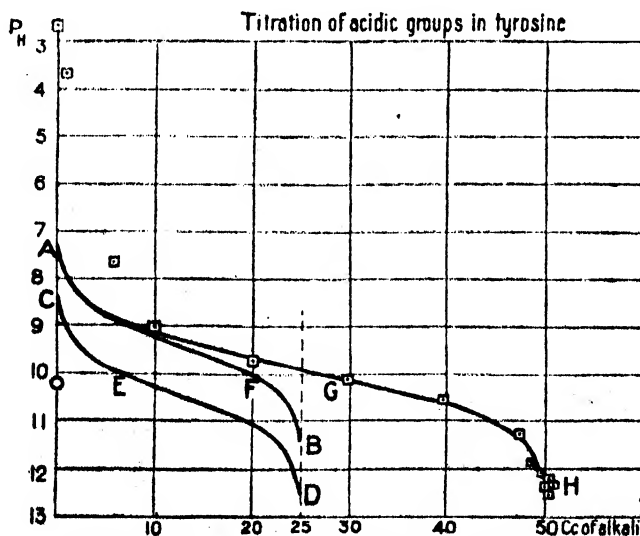


FIG. 13.—Titration of Acidic Groups in Tyrosine.

clear. AB is the titration curve for an acid with  $K_a = 4 \times 10^{-10}$ , CD that for an acid with  $K_a = 4 \times 10^{-11}$  (the two values for the acidic dissociation

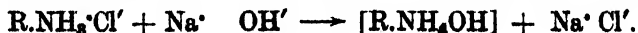
of tyrosine). The amount of N/10 acid *OG* required to bring 25 c.c. of N/10 tyrosine to any  $P_H$ , *O* is the sum of the amounts *OE* and *OF* required to bring 25 c.c. (N/10) of an acid  $K_a = 4 \times 10^{-11}$  and 25 c.c. (N/10) of an acid  $K_a = 4 \times 10^{-10}$  to the same  $P_H$ , *O*. The resulting curve (calculated) is represented by the line *GH*; the experimental values (corrected for blank) are shown by squares.

### *The Titration of Lysine.*

It is possible in a similar way to predict the titration curve for lysine, but the dissociation constants are in this case based on somewhat uncertain data (see p. 451).

It will be convenient to investigate theoretically the titration of one gram-molecule of lysine *dihydrochloride* with three gram-molecules of soda; this will be equivalent to examining the titration of free lysine with both (a) two equivalents of hydrochloric acid (titration of  $(NH_2)_2$ ) and (b) one equivalent of soda (titration of  $CO_2H$ ).

$K_{s2}$ .—Lysine dihydrochloride will be strongly acid, but not quite so acid as the theoretical end-point calculated from the constant  $K_{s2}$  because part of the hydrochloric acid is employed in bringing the solvent to the acid reaction (i.e., the blank correction) in addition to that employed in the neutralisation of the second  $NH_2$  group in lysine. Assuming that  $K_{s2}$  is  $1.1 \times 10^{-12}$ , we should expect on addition of soda to lysine dihydrochloride to obtain a (corrected) titration curve with mid point at  $P_H 14 - \log K_{s2} = 2.04$  and end-point (99 per cent.) at  $P_H 14 - \log K_{s2} + 2 = 4.04$ . This curve will be of the same form as the titration curve of a moderately strong acid of  $pK_a = 2.04$ , but is in reality the displacement curve—corresponding to the dissociation residue curve—of a very weak base, namely, that of  $K_{s2}$  for lysine.



The curve is also identical with that of the true titration curve obtained by the addition of HCl to lysine monohydrochloride, except that the direction of the abscissæ is reversed.

The uncorrected curve will lie slightly lower than the corrected curve, the horizontal difference at any  $P_H$  value representing the amount of acid required for the solvent alone.

Figures were obtained by Tague (*loc. cit.*) showing the change in  $P_H$  caused by the addition of soda to lysine dihydrochloride, but no account was taken of the effect of un-neutralised HCl on the solvent, and the curve was supposed to represent the neutralisation of the carboxyl instead of the amino group.

In fig. 14, AB is the experimental (corrected) titration curve. It corresponds very accurately with a theoretical curve having  $K_{b2}$  value  $1 \times 10^{-12}$ .

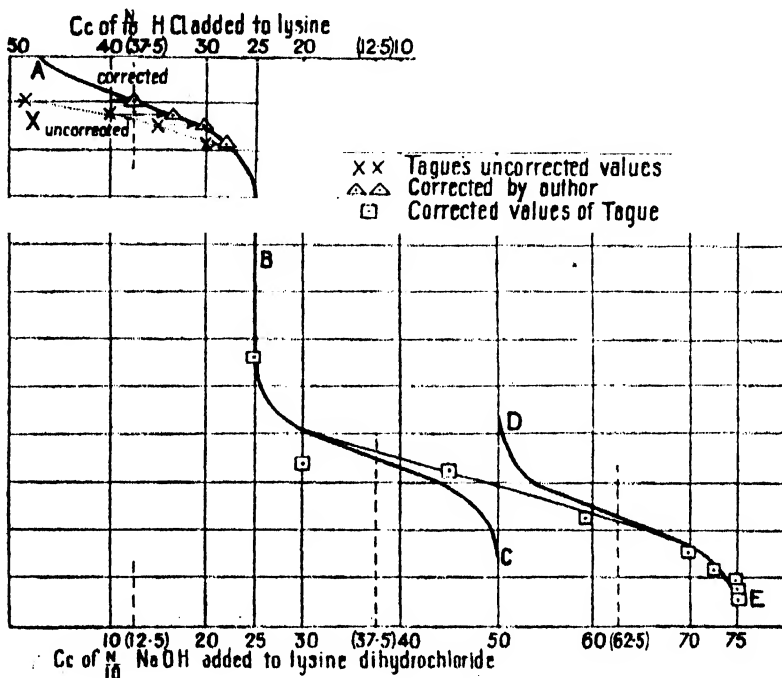


FIG. 14.—Titration of 2 · —NH<sub>2</sub> and —CO<sub>2</sub>H in Lysine.

This again is in good agreement with  $K_{b2} = 1.1 \times 10^{-12}$ , the value determined by Kanitz.

The curve indicates that at  $P_{H2}$ , the weaker of the two NH<sub>2</sub> groups in lysine is 50 per cent. ionized, and that in solutions more alkaline than  $P_{H4}$  the NH<sub>2</sub> is inappreciably ( $< 1$  per cent.) ionized and incapable of acting as a base.

The correction of Tague's experimental values for the effect of solute is shown in Table XV. XB in fig. 14 represents Tague's uncorrected readings.

$K_{b1}$ .—The addition of further NaOH will cause a rapid increase in  $P_H$  until the region is reached in which the more strongly basic of the two NH<sub>2</sub> groups becomes increasingly non-ionized. The curve will then flatten out and trace the displacement (dissociation residue) curve of  $K_{b1}$ .<sup>\*</sup> After the addition of an equivalent of soda the end-point will be reached, at which the NH<sub>2</sub> is no

\* Assuming, of course, for the moment that there is no interference from the overlapping of the carboxyl dissociation-curve, and, for the sake of the theoretical deduction, regarding each as independent of the other.

Table XV.

(a)	(b)	(c)	(d)	(e)	(f)	(g)
c.c. of N/10 soda added to 100 c.c. of solution containing 50 c.c. of N/10 HCl.	c.c. of N/10 HCl remaining.	Resulting Pa.	At total volume.	Correction for 100 c.c. (from curve).	Correction for given volume.	Corrected c.c. of N/10 HCl remaining.
	$(b - 50 - a)$		$(d - 100 + a)$		$(f = de/100)$	$(g = b - f)$
1.5	48.5	1.97	c.c. 101.5	c.c. 11	c.c. 11	37.5
10.0	40.0	2.25	110.0	6	6.6	33.4
15	35	2.46	115.0	4	4.6	30.4
20	30	2.84	120	1.5	1.8	28.2

longer ionized, having been depressed by the more strongly basic NaOH (the point C).

The two  $\text{NH}_2$  replacement curves will be the mirror images of the two  $\text{NH}_2$  neutralisation curves, and will be identical in shape with the corresponding acid titration curves connected by the formula

$$\log 1/K_a = \log K_b/K_w.$$

$K_a$ .—On the addition of still more alkali the  $\text{P}_{\text{H}}$  zone will be reached at which the weakly acidic carboxyl becomes appreciably ionized and the acid titration curve will then be traced. Owing, however, to the similar magnitudes of  $\log 1/K_a$  and  $\log K_{b1}/K_w$ , the two latter curves overlap. In other words, at the commencement of the titration of the carboxyl the more basic of the  $\text{NH}_2$  groups is still dissociated to some extent. At the end part of the titration, near E, the carboxyl is free from the effect of  $\text{NH}_2$ , and by upward extrapolation the curve ED is obtained, showing the titration of the carboxyl without reference to and as if it were independent of the overlapping effect of the basic dissociation. The mid point of the curve DE,  $pK_a$ , is 10.7, giving the value  $K_a = 2 \times 10^{-11}$ , in good agreement with that of Kanitz ( $1 - 2 \times 10^{-11}$ ). The  $K_{b1}$  curve BC has been drawn with  $14 - pK_{b1} = 9.5$  corresponding with  $K_{b1} = 3.2 \times 10^{-5}$  (Kanitz gives  $K_{b1} > 1 \times 10^{-7}$ , amended). The  $K_{b1}$  curve and the  $K_a$  curve have been combined in the single curve BE, which predicts the net

effect of adding alkali to lysine monohydrochloride. Represented by squares are the experimental corrected values of Tague. One reading at  $P_H$  9.57 rather seriously deviates from the theoretical curve. Further readings with lysine are to be desired. The titration-curve represents travelling from top to bottom (1) the back titration of the weaker  $NH_2$ , (2) the stronger  $NH_2$ , and (3) the titration of the carboxyl groups respectively; it may be noted that Tague supposed it to represent (1) the neutralisation of carboxyl, and (2), (3) two hydrochloric acid groups respectively.

("The strongly negative chlorine influences the molecule to such an extent that lysine-dihydrochloride ionizes as a strong acid. Since both amino groups are combined with hydrogen chloride, the carboxyl will be neutralised above the  $P_H$  7.0 point. An excess of hydroxyl ions are necessary, however, to split off the combined hydrogen chloride groups." *Loc. cit.*, p. 184.)

It will be seen from the conclusions reached in this section that the titration-curve is typical of a given amino-acid. By carrying out titration on protein-hydrolysis mixtures after the various separations it will be possible to obtain information concerning the amounts of the different constituents or groups present,\* and it may be expected that such results will be much more nearly quantitative than those obtained in the past, which have often depended on actual separation and weighing of the acids or their derivatives.

The procedure indicated above has further applications in the determination of the acid or alkali binding power of the more complex ampholytes, peptides and proteins. Also, many organic substances containing weakly dissociating groups, and in particular those occurring in fluids of biological importance, are capable of being estimated by these titration methods.

### Part III.—ESTIMATION OF $NH_2$ AND $CO_2H$ BY USE OF INDICATORS.

By employing the results obtained in the preceding sections one can deduce what percentage of the total amino- or carboxyl-groups contained in a given amino-acid solution is neutralised when the solution is titrated to a given  $P_H$ . Hence, by observing the amount of HCl (or NaOH) required to take a solution of an amino-acid to a given hydrogen-ion end-point one is enabled to estimate the total amino (or carboxyl) present, which also serves to define the amount of amino-acid present. Again, if more than one amino-acid is present and if different percentages of each are neutralised at any  $P_H$  value, it

\* See, for example, results obtained with the quinhydrone electrode, p. 466.



is possible to estimate each by titrating to more than one  $P_H$  end-point. [For example, suppose one has a solution containing two bodies A and B, and their respective dissociation curves indicate that at  $P_H$  (say) 8, 50 per cent. of A is neutralised and 1 per cent. of B, and that at  $P_H$  (say) 10, 99 per cent. of A and 50 per cent. of B. Then, by measuring the amount of titrant required to bring the mixture to the two end-points (1)  $P_H$  8 and (2)  $P_H$  10 the amounts of both A and B in the solution can be ascertained. Similarly, if there are *three* substances present, at least *three* titrations must be carried out with three different end-points, and so on.]

The attainment of the desired end-point may be indicated electrometrically without the necessity for a potentiometer, by making contact by means of a salt bridge with a second hydrogen electrode containing a standard buffer solution at the given  $P_H$ , zero deflection of a galvanometer denoting equalisation of potential in the two solutions; or in place of the standard solution a special half cell may be employed as suggested by Pinkhof (21) with single potential equal to that of the end-point of the titration.

Remarkably accurate results have been obtained by the *use of indicators*. Generally, no attempt was made to carry the titration to completion, but acid or alkali was added until the attainment of a pre-determined  $P_H$ , and the burette reading was multiplied by a factor read from the dissociation curve corresponding to the fraction neutralised at that  $P_H$ . As the end-point chosen was therefore generally in the middle flat portion of the titration curve, the addition of titrant caused comparatively little change in the colour of the indicator; and to obtain most accurate results the colour was matched in a comparator against that of a standard consisting of indicator with buffer solution of the desired  $P_H$ . To estimate solutions containing varying concentrations of amino-acid it was found most convenient to add to a standard sample, containing a *known* concentration, the indicator and a given amount of the titrant, and then titrate the unknowns to the same colour. The concentration of amino-acid in the final volume of solution after titration was in each case proportional to the amount of acid or alkali used for the titration. From the known concentration of the standard the absolute amounts of amino-acid present in the various solutions could be determined.

When necessary a blank-correction was applied corresponding to the amount of titrant required to bring water alone to the same final volume and the same  $P_H$ . To keep the blank correction small, N/1 (in place of N/10) acid or alkali was used (with a micro-burette), dilution of the solution being thereby avoided (v. pp. 459-460).

For estimations at acid end-points ( $P_H$  1.2--2.8) Clark and Lubs' Thymol Blue was found convenient; at alkaline end-points ( $P_H$  11.1--12.7) Tropæolin O gave good results.

The following are a few representative readings.

Table XVI.—Colorimetric Estimation of  $NH_2$  by Partial Titration.  
(Solutions Titrated to  $P_H$  2.5 (Thymol Blue).

No.	Description.	c.c. of N/1 HCl required.	c.c. of N/1 HCl calculated from No. 1.	c.c. of N/1 HCl deduced theoretically from $K_a = 5 \cdot 1 \times 10^{-12}$
(1)	20 c.c. of N/10 alanine	1.22 corrected		1.24
(2)	20 c.c. of N/20 alanine	0.62	0.61	0.61
(3)	20 c.c. of N/40 alanine	0.27	0.30	0.31
Titrated to $P_H$ 2.7 (Theoretical mid-point):				
(4)	20 c.c. of N/20 alanine	0.52		0.50

A similar degree of accuracy was obtained in the titration of carboxyl groups. The following readings were obtained by titrating to  $P_H$  9.2 (Thymol Blue as indicator), at which H ion exponent, according to theory—see fig. 10—22 per cent. of the carboxyl should be neutralised.

Table XVII.—Estimation of Carboxyl in Glycine by Partial Titration.

(a)	(b)	(c)
Quantity of glycine taken for titration.	Quantity of N/10 NaOH required to bring to $P_H$ 9.2 (assumed from theory to be 22 per cent. of amount required for complete titration)	Hence, quantity of glycine, found—( $c = b \times 100/22$ )
10 c.c. of N/10 glycine	2.1 c.c.	9.5 c.c. of N/10 glycine.
20 c.c. of N/10 glycine	4.8 c.c.	21.8 c.c. of N/10 glycine

etc.

Tropæolin O may with advantage be employed as an indicator at an end-point of  $P_H$  about 11.5, at which the carboxyl group of glycine and most other amino-acids can for practical purposes be regarded as completely neutralised. An appreciable blank-correction must be applied.

*Titration to  $P_H$  11.6.*

10 c.c. of N/10 glycine require of N/1 NaOH	.. 1.0 c.c. (uncorrected)
blank for 10 c.c. of water	.. 0.05 c.c.
$\therefore$ 10 c.c. of N/10 glycine require of N/1 NaOH	.. 0.95 c.c. (corrected).

*The "Alkalinity" of Histidine.*

There has been difference of opinion among several writers as to whether histidine is a "neutral" or "alkaline" body. The difficulty is cleared up by a consideration of the dissociation curves. Reference to the diagram, fig. 4, will show that at the so-called "true neutral point,"  $P_H$  7 histidine is only very slightly ionized with respect to the stronger of the two basic groups. The second basic group and the acidic carboxyl are both inappreciably ionized at  $P_H$  7. The addition of only a very small quantity of HCl will therefore be sufficient to bring a solution of the free "base" to the neutral point  $P_H$  7. From this point of view histidine appears to possess no alkaline properties.

On the other hand when a little additional HCl is added to make the solution of histidine slightly more acid than  $P_H$  7 it is observed to titrate as a monoacidic base. At  $P_H$  5.76, 50 per cent. of the base is neutralised and at  $P_H$  3.76, 99 per cent.

It is therefore possible to reconcile views such as the following. Foreman ('Biochem. J.,' vol. 14, p. 462) having found that 10 c.c. of M/20 histidine-monohydrochloride required 5.1 c.c. of N/10 soda to neutralise it to phenolphthalein, wrote:—

"The chloride radicle in the pure salt titrated practically quantitatively in aqueous solution. According to Plimmer (1915) histidine is alkaline in reaction. The result does not support this statement so far as phenolphthalein is concerned."

(The colour of phenolphthalein first appears at  $P_H$  8.5, at which the basic dissociation of histidine is perfectly negligible.)

*Estimation of Histidine.*

The determination of the concentration of histidine in solution is found to be a particularly simple operation, because the end-point (99 per cent. neutralisation) for  $K_{b1}$  occurs at about  $P_H$  3.76, where the secondary effect of the HCl on the solvent plays no appreciable part. Hence after the addition of one mol. of HCl to one mol. of histidine, there occurs a very great increase

\* The isoelectric point for histidine calculated from  $K_a$  and  $K_{b1}$  being  $P_H$  7.21.

of hydrogen ion concentration for a very slight addition of acid. In other words the uncorrected titration curve becomes approximately vertical with the completion of the titration. There is, therefore, produced a sharp change in the colour of an indicator having transition point near  $P_H$  3.76. In consequence, histidine may be estimated with an ease and accuracy almost equal to that of the titration of a strong alkali with standard acid; and this in spite of the fact that an aqueous solution of histidine has a  $P_H$  not far removed from that of pure water.

Table XVIII.—Estimation of Histidine.

By titration, as a monoacidic base, to  $P_H$  3.8 (bromphenol-blue as indicator).

	Required, c.c. of N/1 HCl.	Calculated for complete titration, c.c. of N/1 HCl.
20 c.c. of N/10 histidine	2.01	2.0
20 c.c. of N/20 histidine	1.02	1.0
20 c.c. of N/40 histidine	0.53	0.5

*Constants for Tryptophane.*

When the same amounts of N/1 HCl or N/1 NaOH were added to both N/10 *tryptophane* and N/10 *alanine* it was found that approximately the same  $P_H$  resulted with the two amino-acids. This result indicates that the constants  $K_a$  and  $K_b$  for *tryptophane* are of very similar magnitude to those for *alanine*.

SUMMARY.

*Part I.*

The theory of titration is discussed in relation to amphoteric electrolytes with special reference to the amino-acids resulting from protein hydrolysis.

The general theory is shown to hold with considerable accuracy provided a solution containing amino-acids is regarded as a mixture of bases and acids with the same dissociation constants as those of the amino- and carboxyl-groups (or basic and acidic radicles) present.

In the case of the "neutral" *monoamino-monocarboxylic acids* the amino-group is completely dissociated as hydrochloride, etc., at  $P_H$ 's ranging from 0.1 to 0.71, the carboxyl-group at  $P_H$ 's ranging from 10.60 to 11.73. At the "neutral point" ( $\alpha. P_H$ ) the *monoamino-monocarboxylic acids* are therefore inappreciably ionized either as acids or bases.

In the case of the *dicarboxylic-monocamino acids*, glutamic and aspartic, one carboxyl group is completely\* dissociated as sodium salt, etc., at all  $P_H$ 's less acid than 6.4 and 5.8 respectively. The remaining acidic and basic groups are dissociated at more alkaline and acid reactions respectively.

One basic group of the *diamino-monocarboxylic acids*, arginine and lysine, is completely\* dissociated as hydrochloride, etc., at  $P_H$  somewhat alkaline of 7. Similar values are given for other amino-acids and peptides, and it is concluded that amino- and carboxyl-groups may be estimated by titrating to suitable alkaline and acid  $P_H$  end-points, and rules are given.

Dissociation-curves are given for the various amino-acids, etc., from which the percentage neutralised of the different groups at any  $P_H$  may be read.

### Part II.

When titrating feebly basic or acidic groups a "blank-correction" is introduced, corresponding to the amount of standard titrant which must be added to water alone to produce the same final volume and the same  $P_H$ . Figures and curves are given from which the suitable blank-correction may be read.

Applying this correction, the experimental determinations of Sørensen, of Eckweiler, Noyes and Falk, of Tague, and of the author, of the  $P_H$  values of buffer solutions of amino-acids, etc., with varying amounts of acid and alkali, are utilized to show that the experimental titration curves are in very close agreement with those calculated by the mass-law equation from the respective  $K_a$  or  $K_b$  constants determined from conductivity measurements, catalysis, etc.

A method is given whereby the actual uncorrected titration readings (i.e.,  $P_H$  of ampholytes in presence of varying amounts of acid or alkali) may be calculated from the theoretical values.

The use of N/1 (in place of N/10) HCl or NaOH diminishes the blank correction. An increase in the dilution of a solution results in an increased divergence between theoretical and actual (or uncorrected) curves, i.e., in an increase in the blank correction. The latter becomes appreciable as the solution becomes very acid or very alkaline.

When only the earth part of a "simple" titration curve has been determined it is possible to continue it by extrapolation to the end-point and an accurate estimation can be effected.

From experimental titration curves the constants  $K_a$  and  $K_b$  for various

\* More correctly 99 per cent. or over.

amino-acids and dipeptides have been deduced. The results are in good accord with the constants determined by other methods. Several constants due to Kanitz are corrected, and some constants determined for the first time. The titration curves of dipeptides show no evidence of addition of acid or alkali at the peptide linkage, such as has been suggested to occur.

In the case of tyrosine and lysine there is serious overlapping of the several dissociation curves, but by a process of summation the resulting titration curve is deduced and the various parts identified.

The conclusions arrived at in this section may be utilised in the quantitative estimation of amino-acids in the various solutions resulting from protein hydrolysis, for controlling separations, and often for estimating individual amino-acids in complex mixtures. By determining the complete titration curve of any mixture of amino-acids, an estimation can be deduced of each of the following groups:—monoamino-monocarboxylic acids, dicarboxylic-monoamino acids, diamino-monocarboxylic acids.

The use of the quinhydrone electrode is suggested as a convenient and highly accurate method for determining  $\text{NH}_2$  or other basic or acidic groups. The following estimations have been made with its aid:—(1) Single amino-acid (alone or in presence of mineral acid or alkali); (2) total amino groups; and of each of the following, alone or *present in mixtures* of monoamino-monocarboxylic acids: (3) Glutamic acid; (4) Lysine; (5) Histidine; etc.

### *Part III.*

With a knowledge of the percentage ionization of various acidic and basic groups at given  $P_H$  values, it is possible to estimate accurately such groups by titrating to the given  $P_H$  values in the presence of suitable indicators. Examples are given.

Histidine may be estimated by titration with standard  $\text{HCl}$  to  $P_H$  2.5 with thymol blue, one basic group being then completely ionized as hydrochloride. Apparently conflicting views with regard to the basicity of histidine are reconciled.

In Parts IV-VI methods will be described for estimating  $-\text{NH}_2$  and  $-\text{CO}_2\text{H}$  in *alcoholic and formaldehyde solutions*, and the theory of titration will be discussed in relation thereto.

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*Studies in Brownian Movement.—II. The Determination of Avogadro's Number from Observations on Bacteria (Cocci).*

By J. H. SHAXBY.

(Communicated by Prof. S. W. J. Smith, F.R.S.)

[This paper is published in Series A. No. 728.]

*Address of the President, Sir Charles S. Sherrington, at the  
Anniversary Meeting, November 30, 1923.*

At the Anniversary Meeting of the year our minds naturally revert to those of this Fellowship whom the past twelve months have taken from it.

In December last, in his eighty-third year, died CHARLES DOUGLAS HANBURY-TRACY, 4th Baron Sudeley, naval officer and also Member of The Inner Temple. Keenly interested in science, he had given long public-spirited service to the cause of the National Museums, urging on more than one Government and at more than one period critical for their finance, the scientific and educational importance of them to the country.

CHARLES IMMANUEL FORSYTH MAJOR died on March 25. By profession a physician, by inclination a naturalist, he had spent the greater part of a long and vigorous life-time in pursuit of palæontology. To that subject his field work in Italy, Samos, Madagascar, Corsica and elsewhere brought memorable contributions. His collections went in large measure to the national collections. At the British Museum for a time he was engaged in cataloguing the fossil mammals, a chapter of palæontology on which he was a specially equipped authority.

On March 27 died at the Royal Institution, where he had been Fullerian Professor of Chemistry for 46 years, JAMES DEWAR, successor there to Davy and Faraday, and Director of the Research Laboratory which bears their conjoint names. Like them, a master in experimentation, his achievements ensure him a place along with theirs in the tradition of the Institution where he followed them. The Royal Society's Rumford Medal founded by the founder of the Royal Institution, was awarded to him in 1894; in later years he received from the Society its Davy Medal and its Copley Medal. His Bakerian Lecture, delivered in this room twenty-two years ago, he entitled "The Nadir of Temperature." That designation he gave to it recalls how, by evaporating liquid hydrogen under reduced pressure, he obtained hydrogen as a frozen foam and air as a rigid inert solid. The discoveries he made were some of them rich in practical application, yet it was the sheer enrichment of Natural Knowledge which engrossed his life. To a gift of scientific imagination he had added the acquisition of supreme scientific technique. His tempera-



ment was allied to that of the artist ; for him a demonstrative experiment was akin to an artistic creation. By his enthusiasm for science, its possibilities, its responsibilities, and its prerogatives as he saw them, his nature was sometimes stirred in degree difficult for others to understand. Science he certainly loved, and with the ardour of an explorer for an horizon. Looking once into a friend's album I saw that, when asked to supply a sentence for it, Dewar had written : " No familiarity can reconcile our minds to the incredible properties of that something which we call matter." Those words of his own illustrate, I think, one aspect of the spirit in which he pursued research throughout his time.

JOHN VENN, President of Gonville and Caius College, and for many years Lecturer in Logic and Moral Philosophy at Cambridge, died in April in his eighty-ninth year. Probably the best-known of his contributions to science was a suggestive volume entitled " Symbolic Logic." Always devoted to his College, within whose circle almost all his life was passed, much of the work of his later years was given to writing an exhaustive biographical history of it. And besides those of his College he had issued records of much value for the history of the University itself. To those who knew him personally he stood as an attractive personification of length of years, retaining to their end an undimmed interest in the present, and seeing it kindly as wisely through the perspective of a long vista of the past.

CHARLES NIVEN, mathematician, died in May. He had been Professor of Natural Philosophy in the University of Aberdeen for forty-two years. His original contributions to mathematics date, however, chiefly to the time of his earlier Professorship, namely in the Chair of Mathematics at Cork. His work won repute for analytical skill, and he had been elected a Fellow of the Society as far back as 1880.

SYDNEY SAMUEL HOUGH, H.M. Astronomer, Royal Observatory, Cape of Good Hope. In the course of a brilliant mathematical career at Cambridge he showed mathematically how the elastic yielding of the earth caused a lengthening of the period of the latitude variation from 305 to 430 days. He revised Laplace's " Theory of the Tides " and made what Sir George Darwin described as the most important contribution to this theory since the time of Laplace. At the instance of Sir David Gill, he was appointed Chief Assistant at the Cape in 1898 and succeeded Sir David in 1907. He built very well on the foundations which Gill had laid, and made what is probably the most valuable contribution of recent years to the determination of absolute positions of the stars in the sky. He also gave much thought to the Astrographic

Catalogue and made the Cape section a very valuable contribution to this work. The publication was nearly completed when fatal illness removed him at a relatively early age.

HENRY HOYLE HOWORTH died on July 15, at the age of eighty-one. Of varied attainments, he had written extensively on themes archaeological, ethnological and geological; during a lengthy period he had taken an active part in public life.

HENRY HUBERT HAYDEN, geologist, met his death while returning from a mountain ascent in Switzerland. Director of the Geological Survey in India during eleven years, he had been appointed to it in 1910. He was elected Fellow of the Society in 1915. Of outstanding personality on many counts, he was distinguished scientifically perhaps especially for his Himalayan and trans-frontier stratigraphical work. Accompanying Sir Francis Younghusband's expedition to Lhasa, the observations he then made have been described as opening a new chapter in Himalayan geology. After retiring from the Indian Government's service he again, only last year, visited Northern Thibet. His death, at the age of fifty-four, brought an untimely close to the inspiring career of one who was at once geologist, naturalist, and explorer.

In September died JOHN Viscount MORLEY, of Blackburn, prominent in politics and in letters. He had given direct service to Education as Chancellor of the University of Manchester for the last fifteen years.

HERBERT McLEOD, dying last month in his eighty-third year, had been a Fellow of the Society for 42 years. Early in his career he was Lecture Assistant at the Royal College of Chemistry, and had a part in the discovery of the aniline dye, Magenta. Later he was appointed Professor of Experimental Science (afterwards Chemistry) at the Royal Indian Engineering College, Coopers Hill. There, for measurement of low-pressure of gases, he devised the McLeod gauge, long generally in use for such work. The years of patient and sound bibliographical work he gave to the Catalogue of Scientific Papers constitute a special lien on the grateful remembrance of him by the Society. From 1888 onwards he had read the proofs for the Catalogue, and in May, 1902, he undertook the Catalogue's direction; he prepared the subject-index to its papers dating between 1800 and 1900. He had further seen the author catalogue for the period 1883-1900 half through the press when ill-health obliged him to desist from continuance of that work in 1915.

JOHN ALLEN HARKER died on October 10. At an early point in his research career he had carried out, in collaboration with Chappuis, an elaborate comparison of the gas and platinum thermometer scales. Selected subsequently

for the staff of the then young National Physical Laboratory, he became head of the Thermometry Division of that Institution, and he was for some time Chief Assistant of it. From there issued his valued series of papers on high-temperature measurements. During the war he was responsible for the organisation of the work of the Nitrogen Products Committee of the Ministry of Munitions, and was Director of the Research Laboratories of the Inventions Department of that Ministry. He was an active member of the Oxygen Committee of the Department of Scientific and Industrial Research. In the last few years he had taken deep interest in the question of the large-scale use of oxygen-gas as a therapeutic agent. Never robust in health, and sometimes severely taxed physically during the stress of his war work, the uncertainties of his own health seemed little to abate his high-strung activity, and despite illness his scientific keenness and enthusiasm remained always with him.

ARTHUR ALCOCK RAMBAUT died on October 14, in his sixty-fifth year. Some time Astronomer Royal in Ireland, he had for the past six and twenty years been Observer at the Radcliffe Observatory in Oxford. His contributions to astronomical literature were numerous. A catalogue of 1,012 Southern Stars was issued by him in 1887, and in 1906 appeared his Radcliffe Catalogue. With the publication in this present year of a volume of the Radcliffe Observations containing the resulting parallaxes of 2,400 stars he had had the satisfaction of witnessing the completed issue of a main labour of his life.

On the last day of last month died JOHN EDWARD STEAD, metallurgist and chemist. Throughout his career closely associated with the scientific development of the ferrous industries of this country, he had in 1901 received the Bessemer Medal of the Iron and Steel Institution. He had served that Institution as its President as lately as two years ago.

THOMAS PRIDGIN TEALE, of Leeds, died in his ninety-third year on the 12th of the present month. Distinguished as a surgeon he had contributed to the old established renown of Leeds as a school of surgery. His father before him was a Fellow of the Society. Eminent in the actual practice of his profession, he was also well known for inventions and writings regarding practical hygiene, and for his services to the cause of medical education.

At the Anniversary Meeting it cannot be out of place to iterate the main object of the Society's foundation—"the Improvement of Natural Knowledge," by discovery, and as a never-failing means to that end, the furthering of research. A reference made to it at last year's Anniversary dealt particularly with the

funds at the disposal of the Society for assisting that great purpose. To-day, in reviewing, however briefly, the events of the past year, the Society recalls with lively gratitude the noble gift received from one of its Fellows, Sir Alfred Yarrow. It is a gift specially directed towards this same essential aim of the Society's existence. The Society is happy to have enlisted the services of Sir Alfred himself as a member of the Committee of Management of the Fund furnished by his generosity. The terms of his letter accompanying the donation were no less generous and public spirited than the gift itself. The letter is before you in the Report of Council, but let me quote one point from its contents, a suggestion of practical guidance, perhaps specially germane to a line of policy which began to shape itself in the Society last year, and in which this year has seen further progress. The letter stressed "that the money be used to aid scientific workers by adequate payment and by the supply of apparatus or other facilities, rather than to erect costly buildings."

The receipt of this splendid gift was followed at no long interval by the accruing to the Society of the valuable bequest from its past Fellow, the late Dr. Ludwig Mond. The accession of these funds to the means at the Society's disposal for advancing research has enabled, and suggested, systematisation of its provision for that end. Consideration was undertaken of some adjusted scheme whereby the disbursements the Society could hope to make for the furthering of research should keep suitably in sight the whole ambit of the Society's purview of natural knowledge, thus making for advance over a wide scientific front. For such a plan the Foulerton, Messel, Yarrow and Mond Funds, to mention them in their historical sequence, taken in conjunction and following the wishes of their individual donors, lend themselves well. The scope of destination of these Funds extends from Physics, Chemistry and Engineering, on the one hand, through Biology, to, on the other hand, "Medicine and such sciences as are connected with the discovery of the causes of disease and the relief of human suffering." The mere scant enumeration of the circle of the Natural Sciences suffices to show them as a band of brothers, and seeing them as such is to remember their call is for research, and not even solely for Science's sake itself, but for that of humanity as well.

And, in addition to the question of the breadth of field there remained that also of the particular form which help for research might take in order to be best effective in whatever field rendered. The consideration given to this has been very full and careful. It will be recalled that from the Donation Fund and from some other funds of smaller amount, and also through the Committee administering the annual Government Grant, the Society is able

annually to make disbursements, helpful for apparatus and material, in response to applications in regard to particular items of research. Moreover, the Society has of Research Studentships five in addition to the Sorby Fellowship. All and each of these have rendered and are rendering valuable aid to scientific research in their several respective ways. Broadly taken, their destination is to workers of promise in the earlier period of their career; and such workers are thus provided with opportunity for proving the powers of their promise.

This year, in addition to the above, a generous and public-spirited step taken by the Worshipful Armourers' and Brasiers' Company enables the Society to participate responsibly in the management of yet another endowment of somewhat similar scope. Bearing in mind this relatively satisfactory provision already existent for these needs and recognising, further, the far-reaching outside provision available from Governmental and a number of public and private beneficiary sources, to meet requirements of a similar kind, the opinion arrived at after thorough consideration has been that a form of help specially called for, and specially likely to be effective in advancing discovery, would lie in the creation of greater opportunity for fully experienced investigators of already proven first-rate capacity in research. It is felt that increase of opportunity afforded to such investigators is likely to attain, with a prospect of comparative certainty, its recompense in the achievements such investigators will accomplish. To open up facilities for this class of investigator would seem particularly the province of the Society, and one in which its help could pursue required directions with especially whole-hearted conviction, because the Society in virtue of its own organisation has special opportunity for cognisance of the powers and scientific circumstances of representatives of this class of investigator. Over an ample field, and at many points in that field, the Society lives in contact with their endeavours, conversant with work they have already done and often with work they are, in fact, prosecuting, and could prosecute more fully had they increased opportunity for so doing. The desirability for encouragement of research from the Society to take this kind of shape seems enhanced by circumstances of the present time, including as this present time does the likelihood of an immediate future which will be one of anxiety for finding ways and means. In Institutions, University or other, for the most part such investigators occupy positions to which their opportunities for research attach rather as a secondary adjunct to calls of other nature upon their strength and time. Under an institution's financial stress the demand made by it upon members of its staff who have multifold duties other than research,

is likely to be increased in directions away from research. This is a situation of hardship to the investigator and of detriment and mischance to the due advance of science itself.

Institutions, whether University or other, which are seats of learning, show themselves, in instance after instance, desirous for their personnel to prosecute research, but also, in instance after instance, embarrassed to secure to them adequate time for doing so. And yet the research activity of these men—or, for that matter, women—is a main source of that improvement of natural knowledge which it is the Society's great business to promote. A spring of indispensable supply for the production of new knowledge is thus stemmed or curtailed. Therefore, it is felt that the Society by securing, in co-operation with this or that particular institution, ample freedom of time for a distinguished member of the personnel there to undividedly prosecute research, may extend a form of help toward the advance of discovery particularly desirable and welcome. It is felt that by so doing the Society can gear most usefully its own motive help into the general existent running machinery for the production of new scientific knowledge. The hope is, and the belief is, that its action may thus provide exactly a something which other Institutions might have special difficulty in providing. The action it is taking marks a course which, although entered upon tentatively and to be judged finally by experience, is yet inaugurated with the foundation of three research Professorships of the Society. The regulations for these appointments have been drawn up with intention to give the Professors utmost freedom to carry out research in the way dictated by their individual attainment, temperament and inclination. Council have not thought fit to insist that the Professors either shall teach or shall not teach; the sole restriction laid down is that to research shall their main energies be devoted.

At the Anniversary Meeting last year I had the pleasure of referring to the appointment, then literally hardly more than one hour old, of Prof. Starling as Foulerton Professor. This year has seen him Harveian Orator of the Royal College of Physicians and, as regards the Society, entered fully upon the actual activities of the Foulerton Professorship. Now, at this present Anniversary, the pleasurable privilege falls to me of announcing the appointments of Prof. Fowler and Mr. G. I. Taylor to the Yarrow Professorships. I may be allowed here a few words of reference to them. I follow the alphabetical order of their names. Prof. Fowler is known the world over as a spectroscopist whose researches have been of the greatest value to astronomy, to physics, and to chemistry. Entering on science first as a pupil of, and then as an assistant to, Sir Norman Lockyer

his earlier researches were, as that provenance made natural, astrophysical in kind, although the special technique which he developed was a technique of methods purely laboratory. He achieved extraordinary success in identifying lines observed in stellar spectra with lines which he was able to reproduce in the laboratory. He was able thus to assign the lines to their chemical origin; for instance, the origin of the bands which dominated the spectra of what were then described as stars of Secchi's third class had been a mystery for many years. Fowler was able to show that they were due to titanium oxide. He accounted for many of the bands in the sun-spot spectrum by showing that they belonged to "magnesium hydride." Again, he made an interesting study of the spectra of comets. The spectrum of the head had been observed by Donati in 1864 and had been fully studied by Huggins and others. It remained for Prof. Fowler to make a study of the tail spectrum of comets. He noticed first that the observed spectrum coincided with one which had been obtained in the laboratory arising from an impurity in low-pressure hydrogen. Finally, after much effort and laborious work, this spectrum was found to originate in carbon monoxide.

While these are perhaps some of the more striking of Prof. Fowler's successes in the region of astrophysics, he has also done a great deal of highly useful work in adding to our knowledge of the spectra of known terrestrial substances. Special mention may perhaps be made of his study of the spectrum of scandium, which proved to be important both in solar prominences and in sun-spots; of magnesium, in which he discovered new series of spectral lines; of strontium, in which he added several lines to the already known triple series; and of the active modification of nitrogen discovered by the present Lord Rayleigh.

At the time that these investigations were carried out there was no reason to suppose an immediate future of practical importance for the results obtained, but with the advent of Bohr's theory of atomic structure they have been found to provide exactly the material required for full discussion of the new theories of atomic structure, and for the acquisition of new positive knowledge as to the details of atomic mechanism.

Perhaps his success of most striking general appeal has been his direct experimental proof that the so-called  $\zeta$ -Puppi series of hydrogen originate from helium and not from hydrogen at all. This result incidentally provided a striking confirmation of Bohr's theory of the origin of spectra.

In this field of research Prof. Fowler stands unrivalled. Recently he has been examining the changes which take place in the spectra of elements as

one electron after another is removed ; the results obtained are of fundamental importance. His last paper on the "Spectrum of Trebly Ionised Silicon" will still be fresh in the minds of many of our Fellows.

Branches of physical science other than those benefiting by Prof. Fowler's work have formed the field of research of the Society's other Yarrow Professor, Mr. G. I. Taylor, namely, mathematics, engineering and geophysics. Prof. Taylor started his scientific life as an applied mathematician, and the Society is still fortunate in receiving from him frequent mathematical papers on hydrodynamical themes. Before the advent of Mr. Taylor to this field it was almost a foregone conclusion that the results of mathematical research in a large part of hydrodynamics would not be confirmed by experiments ; Mr. Taylor has opened an era in which experiments and analysis give confirmatory results. From abstract hydrodynamics he was led to research in practical problems of geophysics and meteorology. He has a distinguished record in aeronautical science, dating from the time when, acting as Meteorological Adviser to the Air Force, he was led to study the motions of the air, the causes and effects of eddies and the complicated phenomena to which these give rise. The application of much of his work to problems connected with aircraft is very direct. As the result of mathematical calculations he designed a parachute possessing many advantages in practice ; quite recently he has published an important theoretical investigation as to the manner in which the forces on a model aeroplane in a wind-channel are affected by the eddies set up at the channel's mouth. Some contributions by him have proved of high value to the theory of the propeller.

He has been equally successful in the application of mathematics to engineering problems. In collaboration with Mr. A. A. Griffiths, he made use of the fact that the equations which determine the torsion of an elastic bar are identical with those representing the displacement of a thin membrane stretched over a hole of suitable shape when slightly distorted by uniform pressure. By micrometric measurements of the distortion of such a membrane he was able to deduce the torsion stresses inside a bar of specified cross-section, a procedure having practical applications of the greatest importance.

In the last Bakerian Lecture delivered before the Society, Mr. Taylor, in conjunction with Miss Elam, studied the strains in a single crystal of aluminium when stretched to breaking point, using a most ingenious combination of micrometric measurements and X-ray analysis. In this way



he was able to trace the internal motions in the crystal and to explain the striking difference between the fracture of a bar of ordinary metal and that of a single crystal, such as he examined. In this, his most recent work, he has opened up a field which promises to be of far-reaching importance to the science of the strength of materials, and, I venture to think, of great practical value to the working engineer.

The record of both of our new Professors gives every justification for hoping that in the unfettered freedom of the Yarrow Professorships they may find the opportunity for still ampler fulfilment of brilliant work. It is fortunate that they will both continue their researches in the Laboratories from which their outstanding work has issued in the past, and of whose tradition indeed their reputations already are a part.

It is interesting for us to recall that to encourage and enable investigation in this kind of way has been, although only now entering within the Society's power of accomplishment, a near wish of the Society at past times in its history. These Professorships and the policy they indicate are reminiscent of a page of the very preface of the Society's whole story. If we turn to Bishop Sprat's contemporary account of the first years of the Society we see in that volume's frontispiece, freshly with us from Sir Robert Hadfield's recent admirable reproduction of it, the figure of Francis Bacon, along with those of the giver of our Charter, King Charles, and of the first President, Lord Brouncker. Sprat records that to Bacon's "*New Atlantis*" much of the inspiration for the inception and early life of the Society was due. The book had appeared some thirty-five years before the date of our first Charter. In it Bacon had outlined what to many seemed a sort of prototype foreshadowing the Royal Society itself: "a College," his words run, "for the obtaining of knowledge of the causes of things." He described that College's prospective grounds and apartments, and then enumerated its Fellows and their functions.

"Of its Fellows," wrote Bacon, "twelve there be that sail into foreign countries to bring patterns of experiments from all other parts. These we call merchants of light. Then three we have that collect the experiments which are in all books. These we call depredators" . . . "And," he continued, "on our foundation we have three Fellows who all their days try new experiments such as themselves think good. These we call pioneers." To-day, on the completion of its two hundred and sixty-first year of existence, the Society finds itself able to fulfil, and is fulfilling, closely this particular of Bacon's enthusiastic imagining. Three Fellows on its Foundation

to "try all their days new experiments such as themselves think good." Like Lord Bacon we may well designate them pioneers. And also we shall think of them as Yarrow Professor Alfred Fowler, Foulerton Professor Ernest Starling, and Yarrow Professor G. I. Taylor. Let me add how earnestly we wish them all success in their "new experiments such as themselves think good."

And, finally, may I in general terms return once more to summarise that leading motive, which has actuated the launching of these new Professorships. Our Universities and other scientific institutions have been wont, indeed in many cases by force of circumstances are compelled, to regard teaching as the primary occupation of Professoriate and Staff, and to envisage their occupation in research as merely secondary to their occupation in routine teaching. The Society has inverted quite deliberately that order of precedence of professorial function. By this inversion the Society of set purpose desires to recognise research as a definite profession and to advance, and to maintain, the principle that the labourer is worthy of his hire no less when engaged in research than when engaged in class instruction.

And upon this subject yet one word more. Munificent as the gifts are which the Society has received, enabling it to do what it is doing toward this end it has at heart, may we not venture to hope that the funds already to hand for that purpose will prove but the auspicious starting-point for yet others of similar destination. To say this is but to echo the concluding sentence of Sir Alfred Yarrow's<sup>\*</sup> memorable letter. With such aspirations, our desire is that in due course either the Royal Society or other bodies may have it in their power to endow the research of all those individuals whose life ought, in the best interests of the Community, to be devoted to scientific research as the main purpose of their life-career.

Last year, allowing myself a reference to physiology and its progress, I adverted to the high promise for importance implied in the discovery of "insulin" by Drs. Banting and Best in Prof. J. R. Macleod's laboratory at the University of Toronto. Professor Macleod was among the candidates elected to the Fellowship of the Society this spring. Insulin's promise of fruitfulness has in the elapsed twelve months proceeded satisfactorily toward further fulfilment. It would seem prospectively possible that under treatment by insulin the  $\beta$ -cells of the pancreatic islets may be able to re-establish permanently their functional powers, and that in certain cases the treatment may produce not only temporary but lasting relief from the diseased condition. All the more welcome therefore is it to note that inter-

national recognition of their work was last month received by Dr. Banting and Professor Macleod in the form of the award of the Nobel Prize for Medicine for 1922. The success of their work was based on intimate co-operation between physiology and biochemistry—if, indeed, these cognate studies can be considered separate—and the success of their research early in its progress instanced the value of team-work in the best sense of that term. That aspect of it cannot be touched without a word of tribute to the act of comradeship on the part of the two Nobel Laureates themselves in sharing their emoluments from that prize with their two other collaborators, Dr. Best and Professor Collip. And exemplifying the admirable furtherance of a scientific enquiry by organised collaboration, on a wider scale, yet tantamount in fact to team-work, has been the rapid advance made for the year in the whole study, practical and theoretical, of insulin and insulin-treatment under the earnestness and wisely-directed energy of the Medical Research Council.

And, leaving insulin to its success, let me turn finally to mention of a further richly deserved international recognition won by the work of another Fellow of our Fellowship, Professor A. V. Hill, of University College, London. The award to him of the Nobel Prize in Medicine is specially and universally gratifying to physiologists. His investigations have dealt with the thermodynamical processes underlying muscular contraction, and underlying also the muscular restoration after contraction, enabling contraction to repeat itself again. Professor Hill's elucidation of this whole problem, one which has long occupied and taxed the powers of many eminent physiologists, is as far-reaching in outlook as it has been masterly in conception and in execution.

I now proceed to the presentation of the Medals. The Copley Medal is awarded to Prof. Horace Lamb.

For forty years Professor Lamb has been recognised as one of the most prominent and successful workers in applied mathematics in this country. He is the foremost authority on hydrodynamics, not only in this country but the world over. His treatise on this subject, now in its 4th edition, has long been the standard textbook. Its first edition systematised for the first time the science of hydrodynamics as that emerged from the researches of Stokes, Kelvin and others. With successive editions the book has increased in range and size, and included more and more of the author's own researches, until it finally takes rank among the classical treatises in the domain of mathematical physics.

Professor Lamb's scientific activity, originally centring around the subject

of hydrodynamics, has radiated thence into most branches of physical science. His earlier papers were concerned largely with investigations of solutions of the wave-equation

$$\nabla^2\phi = \frac{1}{V^2} \frac{d^2\phi}{dt^2},$$

especially with reference to spherical boundaries; they have exercised much influence on developments regarding elasticity and electricity, as well as hydrodynamics. The directness and elegance of his mathematical investigations are no less conspicuous than is their close contact with actual phenomena. His work contains much that is typical of the best qualities of British Applied Mathematics. He may be regarded as the outstanding representative to-day of the school founded by Stokes, Kelvin, Clerk-Maxwell, and Rayleigh.

In recent years he has made important contributions to seismology, the theory of tides, and other branches of geophysics. The breadth of his scientific knowledge, along with the soundness of his judgment and clearness of his vision, have made him a highly valued member of many technical Committees both during the war and subsequently. Specially perhaps should be mentioned the assistance he has given of recent years to the Aeronautical Research Committee. Mathematical questions involved in the flow of air round aircraft, in the action of propellers, and the stresses in aeroplane structure, are of fundamental importance, but are exceedingly difficult; and here, as elsewhere, Professor Lamb's mathematical skill and power of clear exposition have proved of the highest value. By his personal qualities no less than by his outstanding powers as a teacher and investigator he has, for all in contact with him, been a great influence.

A Royal Medal is awarded to Prof. Charles James Martin.

Professor Martin is distinguished for contributions both to physiology and to pathology. Investigating snake venoms he differentiated two groups in virtue of their action, one nervous, the other, so to say, humoral. His work on heat-regulation in monotremes threw light on the evolution of the thermotaxis of warm-blood animals. More recently his researches have lain in the colloidal chemistry of proteins, and in protein-metabolism. A series of experiments carried out by him in collaboration with his colleague, Mr. Robison, on the minimum nitrogen expenditure of man and the biological values of separate kinds of protein-food stands as one of the most valuable contributions yet made to a subject as difficult as it is important. Besides these personal researches at first-hand, Professor Martin has influenced

inspiringly the course of research in medicine in this country. He was a member of the Plague Commission which organised the researches finally determining the mode of transmission of that disease. He was Chairman of the War Office Committee on Anti-typhoid Inoculation. As Director of the Lister Institute he has contributed to many investigations, in addition to those actually issued in his name. Thus he has been intimately associated with the enquiry into the influence of accessory food factors of diet in the prevention and remedying of "deficiency" diseases, such as scurvy and rickets, an enquiry the success of which may be regarded as one of the recent triumphs of preventive medicine. His abilities have been ever at the call of his colleagues, and in the most public-spirited way devoted to the service of the community in general.

A Royal Medal is awarded to Sir William Napier Shaw.

In the great advances made during the last twenty-five years in the science of meteorology, Sir Napier Shaw has been amongst the foremost pioneers. During his twenty years' administration at the Meteorological Office that Office saw three marked steps forward: two of these have been changes in its quarters; the third and greatest has been the change in outlook of the work of the Office, whereby it assumed, under Sir Napier Shaw's stimulating influence, the character of a scientific institution for the interpretation of meteorological phenomena. With the assistance of his scientific staff, he has developed the physical and dynamical aspects of the subject, and has done much to concentrate attention upon the thermodynamics of meteorology, wherein the motions of the water-laden air are interpreted as the action of a thermodynamic engine. By his personal researches and especially in his capacity as President of the International Meteorological Committee he has rendered great services to all branches of the science. He has encouraged the co-ordination of international effort in the study of the meteorology of the globe. He has emphasised the need for systematic treatment of the phenomena in terms of units, in line with the established methods of physics and thermodynamics. His contributions to knowledge of the air and its ways have been largely responsible for changing the basis of meteorology from one of empiricism to one of science. I may mention his investigation of the "Life History of Surface Air Currents," which changed completely the conception of the motion of air in cyclones and anticyclones; his pioneer study of the effect of weather on crops; and his more recent work on the general circulation of the atmosphere which has introduced the notion of an atmosphere largely rigid to vertical motion and

the importance of applying thermodynamical considerations to the atmosphere treated as a whole.

The Davy Medal has been awarded to Prof. Herbert Brereton Baker.

Prof. Baker's researches in various fields of chemical investigation, his examination of highly purified tellurium from various sources for the possible presence of higher members of the same group of elements, and the redetermination of its atomic weight, are of outstanding merit. It is, however, his remarkable researches on the influence of traces of water in modifying chemical change, whether of the nature of combination or of decomposition, which constitute perhaps his especial distinction. For the last forty years he has carried on continuously a series of experiments characterised throughout by extreme insight, patience and manipulative skill. The results obtained by complete drying were as remarkable as they were unexpected, because they were in direct opposition to those which followed careful drying by usual methods. For example, he showed that ammonia and hydrogen chloride could be dried so thoroughly that they would not combine, and that ammonium chloride, when completely dried, could be vaporised without dissociating into its constituents. Still more striking was the demonstration that silver could be fused in an atmosphere of pure, dry hydrogen and oxygen without explosion, though explosion followed instantly upon the admission of a trace of water vapour.

The bearing of Prof. Baker's researches on theories of chemical change is as important as his conclusive experimental demonstrations of the phenomena themselves.

The Hughes Medal is awarded to Dr. Robert Andrews Millikan.

Dr. Millikan has long been regarded as one of the most skilful experimenters in physical science. He is awarded the Hughes Medal especially for his determinations of the electronic charge  $e$  and of Planck's constant  $h$ . When physicists were still ignorant of the value of the electronic charge to within 5 per cent., Dr. Millikan, by a method of the utmost ingenuity, arrived at the value  $4.774 \times 10^{-10}$  E.S.U., for which he claimed an accuracy of one part in a thousand, a claim which has stood the test of time. His determination of  $h$  was not only remarkable in itself, but was of still greater value as finally vindicating the Einstein-Bohr view of the nature of the photo-electric phenomenon.

*The Titration of Amino- and Carboxyl-Groups in Amino-Acids, Polypeptides, etc. Parts IV-VI.—Estimations in Presence of Formol and Alcohol.*

By LESLIE J. HARRIS.

(From the Biochemical Laboratory, Cambridge.)

(Communicated by Prof. F. G. Hopkins, F.R.S.—Received May 24, 1923.)

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PART IV.—THE SÖRENSEN AND FOREMAN METHODS FOR ESTIMATING CARBOXYL.

In the well-known formol titration method of Sørensen (22), neutralised formaldehyde is added to the solution of the amino-acid (or other compound containing  $-\text{NH}_2$ ) and standard alkali run in until the production of a red colour with penolphthalein.

An explanation of this method that has often been advanced in text-books and elsewhere is that the original amino-acid is neutral because the basic  $-\text{NH}_2$  neutralises the acidic  $-\text{CO}_2\text{H}$ , and that with the addition of formaldehyde the basic character of the  $-\text{NH}_2$  is destroyed, with the result that the acidic  $-\text{CO}_2\text{H}$  is free to be titrated.

This view is not altogether correct. A neutral amino-acid is so because the  $-\text{NH}_2$  and the  $-\text{CO}_2\text{H}$  are neither appreciably ionised\*—i.e., functioning neither as base nor acid—in neutral solution. The carboxyl begins to function appreciably as an acid only when the solution becomes alkaline, the amino group as a base when the solution becomes acid. The cause of the neutrality of a monoamino-monocarboxylic acid is quite distinct from the cause of the neutrality of, say, a mixture of potash and hydrochloric acid, or of lysine and glutamic acid. In the latter case both lysine and glutamic acid are highly ionised in neutral solution, as a monoacidic base and a monobasic acid

\* See Part I, fig. 1.

respectively. In the presence of equivalent concentrations of each the respective polarities are compensated and a neutral solution results. A solution of potassium chloride on the one hand consists of a mixture of electro-positive potassium and electro-negative chloride ions; a neutral solution of a mono-amino-monocarboxylic acid on the other hand consists of neutral undissociated molecules of the ampholyte.\*

The carboxyl groups in amino-acids generally become neutralised to completion in the neighbourhood of  $P_H$  11.75; after the addition of formaldehyde neutralisation becomes complete at about  $P_H$  8.7. Both from this raising of the end-point and from the magnitude of the  $P_H$  values resulting after the addition of various quantities of alkali to amino-acid-formalin mixtures, it is concluded that the Sørensen titration method depends on the formation of methylene-amino-acid derivatives having dissociation constants,  $K_a$ , about one-thousand times greater than the constants of the amino-acids from which they are derived. An average round value of  $K_a$  for an amino-acid is  $2 \times 10^{-10}$ , that suggested for the methylene derivative is  $2 \times 10^{-7}$ .

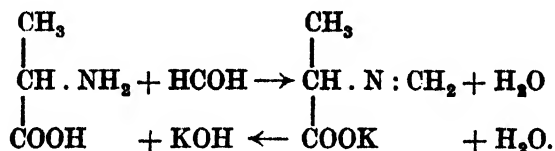
If this explanation is the correct one, it is to be expected that those amino-acids which in aqueous solutions have end-points more alkaline or less alkaline than the normal (owing to less or greater values of  $K_a$ ), will in formol titration show a corresponding shifting of the end-points. This is found to be the case. Lysine has an abnormally low  $K_a$  value ( $1.2 \times 10^{-11}$ ) and a correspondingly highly alkaline end-point ( $P_H$  13.12.7) when titrated in water. Accordingly in formol solution when taken to the normal end-point ( $P_H$  8.8) it was found that only 86 per cent. equivalents of alkali had been added, at  $P_H$  9.1, 93 per cent. equivalents, at  $P_H$  9.5, 97 per cent. equivalents. These readings are in admirable agreement with those required for an acid with  $K_a = 1 \times 10^{-8}$ , the value deduced for the methylene-amino-acid by increasing  $K_a$  for the unmethylated acid ( $1 \times 10^{-11}$ ) one thousand fold. Again,  $K_a$  for arginine is negligibly small; in aqueous solution it exhibits no acid properties. As might, therefore, be presumed it fails to titrate also in the formol method. Sørensen observed this and remarked on "die Passivität der Guanidingruppe." As a third instance may be cited, the behaviour of tyrosine. This acid in aqueous solution combines with a second equivalent of alkali if the titration be carried to a highly alkaline end-point ( $P_H$  12.4), owing to the phenolic group. Similarly in formol solutions, on carrying the titration beyond the ordinary  $P_H$  end-point there is evidence of further combination with alkali:

\* Cf. however, Bjerrum's theory of the *zwitterion*, Part I, p. 442.



at  $P_H$  8.8 there were required 106 per cent. equivalents; at  $P_H$  9.1, 109 per cent.; and at  $P_H$  9.5, 137 per cent.\*

It must be remembered at the same time that the methylene-amino-acid derivative is stable only in the form of its alkali salt. The free methylene-amino-acid tends to revert to the more feebly ionised unmethylated amino-acid. This was realised by Schiff and by Sørensen, who gave the following equation



#### *Titration of Carboxyl in Alcoholic Solution.*

Vorländer (1905) (23), observed the effect of alcohol on certain ampholytes—dimethylantranilic acid, “alkyl-phenylglycin-*o*-carbonsäuren,” “anildiessig-*o*-carbonsäure,” and showed that glycine could be titrated with sodium alcoholate solution in the presence of a high concentration of alcohol. Birckner (1919) (24) observed that “amino-acids, which in aqueous solution are nearly neutral to phenolphthalein, react distinctly acid in the presence of alcohol.” Foreman independently making the same observation applied it to the volumetric estimation of amino-acids (1920) (25), thereby providing the second of the two methods that have hitherto been available for titration of carboxyl in these bodies. A very similar method was described by Willstätter and Waldschmidt-Leitz in 1921, and applied to the determination of amino-acids and peptides (26).

Foreman found that the increase of acidity of amino-acids on addition of alcohol was paralleled by the loss of alkalinity of ammonia and amines when titrated to phenolphthalein; and came to the following conclusions:—

“Ammonia, primary, secondary and tertiary amines and basic methylene derivatives of secondary amines do not form *ionisable compounds with phenolphthalein* in alcoholic solutions containing water, if the concentration of alcohol is sufficiently high.”

“The behaviour of amino-acids in alcoholic solutions on adding potash is comparable with that of an ammonium salt under the same conditions, the

\* Histidine appears to provide an exception to the rule, since it requires a specially alkaline end-point, although its dissociation constant as given by Kanitz is not specially low. In this case, however, an imino-methylene derivative is formed which is basic in aqueous solution, and capable of neutralising part of the acidic carboxyl group.

$\alpha$ -amino-group and ammonia being liberated from the 'internal salt' and the ammonium salt respectively and the potassium salts of the acid radicles formed. With its basic  $\alpha$ -amino-group liberated from the 'internal salt' the amino-acid can be regarded as a true substituted ammonia which would be expected to behave similarly to ammonia and the simple amines in forming no compound with phenolphthalein in alcoholic solutions."

According to Vorländer, also, an inner salt is formed of the nature of a quaternary ammonium salt, which prevents titration in water with caustic alkali. Willstätter, however, supposes that the salt  $\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CO}_2\text{K}$  is a base in aqueous solutions but not in alcoholic. ["Das Salz  $\text{NH}_2 \cdot \text{CH}_2 \cdot \text{COOK}$  ist in wässriger Lösung eine Base (wie Methylamin) in alkoholischer Lösung, aber gibt es gleichwie Ammoniak keine Hydroxyl-Ionen."] To this it must be replied that the difference is one only of degree—in aqueous solution the  $P_H$  of an N/10 solution of the salt is about  $P_H$  11.73, in alcoholic solution it is about  $P_H$  8.8; and, further, the alkalinity in each case is due to the presence of potassium ions from the highly dissociated salt and not to ionisation of the  $\text{NH}_2$  group which remains undissociated in alkaline solution (fig. 1, p. 442 *supra*).

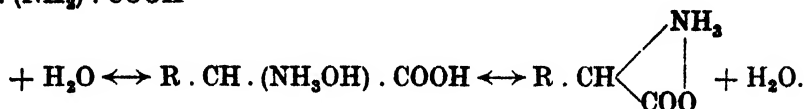
From the point of view of the theory of titration the addition of alcohol must be regarded as causing a rise in the value of  $K_a$  of the amino-acid. The increase is much the same as that produced with formaldehyde but is a shade greater. The effect on the amino-group on the contrary is to diminish the value of  $K_b$  by a very slight but detectable amount. Amino-acids, it is found, require very slightly less acid to titrate to a given  $P_H$  after the addition of alcohol than before.

This result is in one sense surprising because the general effect of adding alcohol to electrolytes is to cause a decrease in acidic or basic dissociation constants. Thus Schidrowitz (27) added alcohol to acetic and hydrochloric acids, and found that a greater concentration of acid was required to bring about the colour change with a given indicator than in the absence of alcohol. Similar conclusions were reached by Lapworth (28) and Jones. The effect of alcohol appears to leave unaffected the  $P_H$  range of the indicator, but to decrease the concentration of H or OH ions produced from a given acid or base.\* The effect of alcohol and formaldehyde on amino-acids is perhaps, however, in accordance with a rule stated by Walker (29) that "a substituent which increases the acid constant decreases the basic, and *vice versa*." An electrometric study of the titration curves of amino-acids in the presence of

\* The evidence for this view is presented in Pridesaux's 'Theory and Use of Indicators,' (16), pp. 177-179.

alcohol and formaldehyde should repay the trouble, but as has been shown by Newbery (30) the determination of  $P_H$  in alcoholic solutions presents considerable difficulties. Recently Hildebrand and co-workers (31) have titrated acids in alcoholic solution by the conductometric method.

Little can be said regarding a chemical change accompanying the rise in  $K_a$  consequent upon the addition of alcohol. It should be stated that the deduction of a value for the acidic constant in water is based on the assumption that an aqueous solution of an amino-acid contains a constant proportion of the three types of molecules, hydrated, unhydrated, and internal salt (see p. 441). A change in this proportion would involve a change in  $K_a$ . Hence it is quite possible that the addition of alcohol or change of solvent may upset the equilibrium



#### Part V.—MODIFICATION OF THE FOREMAN TECHNIQUE.

The method advocated by Foreman for estimation of amino-acid is described as follows :—

“ When aqueous-alcoholic solutions of certain amino-acids *containing about 85 per cent. alcohol are titrated with standard alcoholic potash*, the amino- or imino-groups liberated from their ‘internal salt’ combinations resemble ammonia and the amines in showing no basicity to phenolphthalein, and the carboxyl-groups are accurately estimated.

“ Other amino-acids, more particularly dibasic amino-acids and proline, give low results when titrated in alcohol under these conditions, possibly owing to loose combination of alcohol with a carboxyl-group or loose condensation. *The subsequent addition of formaldehyde or acetone*, however, results in a disturbance of the equilibrium, so that the carboxyl-groups titrate quantitatively.”

According to Willstätter, the titration of amino-acids is to be carried out in 97 per cent. alcohol; polypeptides can be titrated in 40 per cent. alcohol. By titrating first in 40 per cent. alcohol then in 97 per cent. alcohol an estimate can be made of both total polypeptides and total amino-acids in a mixture.

#### *The New Titration Method in Alcohol.*

It has been found by the present writer that those amino-acids which give a low reading when titrated in 85 per cent. alcohol according to Foreman's

directions, titrate quantitatively when for phenolphthalein is substituted *thymolphthalein*, an indicator possessing a more alkaline transition point, i.e., an indicator with a smaller  $K_a$  value.\* Using thymolphthalein in place of phenolphthalein a smaller concentration of alcohol is sufficient to obtain quantitative results, and alcoholic potash may be replaced by aqueous soda. Further, using thymolphthalein there is an increase in the sharpness of the end-point colour change; in the case of phenolphthalein there is a gradual appearance of a very faint pink colour with a number of amino-acids, due to the fact that the indicator's " $P_H$  range" is reached before the attainment of the virtual end-point of the titration, viz., on the latter part of the titration curve where it has not yet assumed an approximately vertical direction.

#### *Analytical Details.*

A concentration of 75 per cent. to 80 per cent. of alcohol was found ample. To 5 c.c. of the amino-acid solution, 50 c.c. of alcohol (97 per cent.) were added and 4 or 5 drops of thymolphthalein (B.D.H. indicator) and N/10 soda run in until the attainment of a faint but quite definite blue colour. No addition of formol is required. A small correction may be applied for the amount of standard soda necessary to produce a blue colour in a blank consisting of the same volume of water alcohol and indicator in the absence of amino-acid.

In the case of tyrosine it was found that about 28 per cent. of the weakly acidic phenolic radicle titrated in addition to the carboxyl when taken to the thymolphthalein end-point. In this case, therefore, the addition of alcohol augments the acidity of both the carboxyl and phenolic groups.

Other amino-acids titrated quantitatively for acidic groups (the carboxyl in arginine exhibiting no acidic properties). An accuracy of 99 per cent.† is obtainable, the errors in the following results being those associated with readings of burettes, etc., rather than any systematic errors in the method.

In dealing with a coloured solution such as lysine-picrate a control was taken containing no indicator, but otherwise identical with the solution under titration and the same amounts of soda run into the control, the end-point being determined by the appearance of a difference in tint between the two solutions.

\* Clark gives the  $P_H$  ranges 8.3 — 10.0 for phenolphthalein, 9.3–10.5 for thymolphthalein.

† Except perhaps in the case of aspartic acid, where the reading is 97½ per cent. of the theoretical; but only 70 per cent. with phenolphthalein.

Table XIX.

	c.c. of N/10 NaOH required.		
	Titration in alcohol to thymol-phthalein (corr.)	Titration to phenol-phthalein in same concentration of alcohol.	Theoretical.
No.			
1. Glycine, 5 c.c., M/10; + 50 c.c. alcohol	5.0	4.3	5.0
2. Alanine, 5 c.c., M/10; + 50 c.c. alcohol	4.95	—	5.0
3. Valine, 5 c.c., M/20; + 50 c.c. alcohol	2.4	2.25	2.5
4. Leucine, 5 c.c., M/10; + 50 c.c. alcohol	4.9	4.4	5.0
5. Cystine, 0.0801 gm. + 50 c.c. alcohol	5.05 (a)	5.0	5.0
6. Phenylalanine - hydrochloride, 0.1008 gm. + 50 c.c. alcohol	10.05	10.0	10.0
7. Tyrosine, 0.0906 gm. + 50 c.c. alcohol	6.4 (b)	5.3	5.0 for monobasic acid; 10.0 for dibasic acid; 6.4 for 28 per cent. of 2nd and 100 per cent. of 1st dissociations.
8. Tryptophane, 0.102 gm. + 5 c.c. hot water + 50 c.c. alcohol	5.0	4.6	5.0
9. Aspartic acid, 5 c.c. M/10 + 50 c.c. alcohol	9.75	7.0	10.0
10. Glutaminic acid hydrochloride, 2 c.c. M/10 + 50 c.c. alcohol	5.9	5.0	6.0
11. Histidine-hydrochloride-hydrate, 2 c.c. M/20 + 50 c.c. alcohol	2.0	1.9	2.0
12. Lysine picrate, 5 c.c. M/40 + 50 c.c. alcohol	2.5	2.45	2.5
13. Arginine, 0.080709 gm. + 4 c.c. water + 50 c.c. alcohol	0.05	0.05 (corr.)	0.0
14. Mixture consisting of—			
1 c.c. M/10 glycine .....	5.8	4.6	6.0
1 c.c. M/10 leucine .....			
2 c.c. M/20 valine .....			
1 c.c. M/10 glutaminic acid hydrochloride			
+ 50 c.c. of alcohol .....			

(a) Determined by dissolving cystine in 8 c.c. of N/10 NaOH and back titrating with N/10 HCl.

(b) Determined by dissolving tyrosine in 9 c.c. of N/10 NaOH and back titrating with N/10 HCl.

A blank correction (averaging 0.2 c.c.) may also be applied to the values for titration to phenolphthalein, causing a still greater divergence from the theoretical values.

*Low Readings in Foreman's Phenolphthalein Method corrected by Titrating in the Hot.*—The fact that certain amino-acids give a deficient titration reading

towards phenolphthalein in alcoholic solution indicates that while  $K_a$  has been considerably augmented above its value in aqueous solution by the addition of alcohol, the increase in  $K_a$  has not been sufficient to bring the titration end-point to the region of  $P_H$  8.5 (phenolphthalein transition zone). A more nearly quantitative estimation will be possible if  $K_a$  is still further increased as by the addition of more alcohol up to 98 per cent. With the addition of more and more alcohol the end-point for complete titration becomes less and less alkaline, denoting a continuous increase of  $K_a$ .\* For practical purposes the presence of 98 per cent. of alcohol in the titrated fluid implies a very considerable dilution and titration with alcoholic potash and a great loss in the convenience of the method.

As is well known the activity of an acid increases with the temperature. Alcoholic solutions of amino-acids were therefore heated in order to bring about an increase in  $K_a$ , and as was expected certain ampholytes which gave deficient readings in the cold titrated practically quantitatively in the hot.

*Effect of Heat on Titration to Phenolphthalein.*

5 c.c. of M/10 alanine + 50 c.c. of alcohol.

At 17° required 4.2 c.c. N/10 NaOH for very faint pink end-point.

50°	„	4.45	„	„	„	„
60°	„	4.5	„	„	„	„
70°	„	4.8	„	„	more distinct pink end-point.	

Apparently the effect of heat, as of alcohol, on the dissociation of the indicator (itself a weak acid) is insignificant compared with the effect on the amino-acid.

In the earlier experiments, using *phenolphthalein*, the present writer also found it convenient, in slight modification of the Foreman method, to determine amino-acids by adding both alcohol and a small volume of neutralised formol to the solution and then titrating with aqueous N/10 NaOH to phenolphthalein. An advantage of this procedure is that a very much less concentration of alcohol is necessary than when the first titration is carried out in the absence of formol which is subsequently added to increase the reading. Great diluting of the solution is thereby avoided. Further, the replacement of N/10 alcoholic potash by aqueous N/10 soda excludes errors due to evaporation of the titrant, and there is no longer the necessity of

\* The fact that polypeptides—whose  $K_a$  values in aqueous solution are greater than those of the amino-acids—may be titrated in a lower concentration of alcohol is further evidence for the correctness of the Author's theory.

preparing anhydrous alcohol which was required for making the N/10 alcoholic potash.

Just as the addition of formol is advantageous in titration of alcoholic solutions, so also I have found that the converse is true. Amino-acids which give deficient titration results in Sørensen's formol method, titrate quantitatively with the addition of small amounts of alcohol.

#### Part VI.—ESTIMATION OF $-\text{NH}_2$ IN ALCOHOLIC AND FORMALDEHYDE SOLUTION.

The action of various solvents on amino-acids has been investigated in an endeavour to produce a rise in  $K_b$  for the amino-group, similar to the rise in  $K_a$  for the carboxyl-group produced by the presence of alcohol or acetone. Could such a solvent be found, the amino-group would be completely titrated with standard acid at a much less acid end-point than is necessary in aqueous solution, and the attainment of the end-point would be shown by the indicator undergoing a sharp change. Although the search has not led to the discovery of a suitable solvent, a simple method has been evolved which may be used in combination with the modified Foreman titration for the estimation of amino- as well as carboxyl-groups in amino-acid solutions.

##### *The Method.*

1. Carboxyl is first estimated by adding alcohol (80 per cent. of the final total volume) and thymolphthalein to the amino-acid solution and titrating with N/10 soda until a blue colour appears.

2. To the same solution methyl-red is now added and N/10 HCl run in until the indicator assumes an orange colour\* (which may be matched against the colour of this indicator in a solution of  $P_H$  5.2–5.6). The amount of HCl required is equivalent to the total  $-\text{NH}_2$  present.

Carboxyl and one amino-group in arginine fail to titrate. A separate blank correction is to be applied to each of the two titrations—(1) the amount of N/10 NaOH required by the water-alcohol-thymolphthalein mixture when no amino-acid is present, and (2) the amount of N/10 HCl required in the back titration of the same mixture to methyl-red.

In the presence of strong acid or alkali the first titration to thymolphthalein is a measure of carboxyl *plus* strong acid or *minus* alkali, the back titration to methyl-red indicates solely the amino-groups.

\* The thymolphthalein becomes colourless on addition of the first drop of acid and does not interfere with the methyl-red.

The method may be applied to distinguish between the various types of amino-acid,\* and between a salt and the free amino-acid; the ratio *titration to thymolphthalein : back titration to methyl-red* differs in the various cases (as shown in Table XX).

Similar information may of course also be obtained by a preliminary titration in water to  $P_H$  6 to 7, the amount of standard acid (or alkali) here required denoting the excess of amino- over carboxyl-groups (or the excess of carboxyl- over amino-groups). But the advantage of the method described above is that a simple and rapid determination of  $-NH_2$  and  $-CO_2H$  may be made on the same sample by direct titration.

#### *Titration to Phenolphthalein ; Effect of Adding Formol.*

The estimation of  $-NH_2$  in alcoholic solution by back titration was first attempted using phenolphthalein for the carboxyl titration, before the advantages of replacing this indicator by thymolphthalein had been investigated.

When dealing with solutions containing only the monoamino-monocarboxylic acids (neutral ampholytes) and the monoamino-dicarboxylic acids (acid ampholytes), neutralised formol may be added in addition to alcohol in order to raise the value of the carboxyl titration (to phenolphthalein). This is not permissible when the solution contains the basic ampholytes (histidine, arginine, lysine) or tryptophane, the acid methylene derivatives formed seriously lowering the  $-NH_2$  estimation (back titration to methyl-red).

In the absence of formol, however, the carboxyl titration is liable to be low in the case of the neutral and acid ampholytes, and in consequence there is a corresponding deficiency in the  $-NH_2$  estimation (by back titration). The error may be averted by titrating a full red to phenolphthalein: the exact amount of standard acid required for this purpose may be accurately determined—viz., that required in the titration of a second sample containing both alcohol and formol. The back titration to methyl-red of the formol-free solution will then give an accurate indication of the  $NH_2$ . The method described above using thymolphthalein in place of phenolphthalein is simpler and more accurate.

#### *Explanation.*

The reactions occurring in titrating the various types of amino-acids in alcoholic solution (1) to thymolphthalein and (2) back to methyl-red will be briefly considered.

\* And hence also in determining the purity of an amino-acid, e.g., to detect the presence of a diamino-acid in a sample of a dicarboxylic acid.



Table

Estimation of carboxyl and amino of amino-acids in alcoholic solution

		Titration with N/10 NaOH to th.ph.	
(1) Monoamino - mono - carboxylic acid.	$\begin{array}{c} \text{CO}_2\text{H} \\ \diagup \\ \text{R} \\ \diagdown \\ \text{NH}_2 \end{array}$	$\xrightarrow{+\text{NaOH}}$	$\left[ \begin{array}{c} \text{CO}_2- \\ \diagup \\ \text{R} \\ \diagdown \\ (\text{NH}_2) \end{array} \right]' \text{Na}^{\bullet}$
(2) Dicarboxylic-mono-amino acid.	$\begin{array}{c} \text{CO}_2\text{H} \\ \diagup \\ \text{R} \\ \diagdown \\ \text{CO}_2\text{H} \\ \diagup \\ \text{NH}_2 \end{array}$	$\xrightarrow[\text{NaOH}]{+\text{NaOH}}$	$\left[ \begin{array}{c} \text{CO}_2- \\ \diagup \\ \text{R} \\ \diagdown \\ \text{CO}_2- \\ \diagup \\ (\text{NH}_2) \end{array} \right]'' \text{Na}^{\bullet}$ $\text{Na}^{\bullet}$
(3) Diamino - monocarboxylic acid	$\begin{array}{c} \text{CO}_2\text{H} \\ \diagup \\ \text{R} \\ \diagdown \\ \text{NH}_2 \\ \diagdown \\ \text{NH}_2 \end{array}$	$\xrightarrow{+\text{NaOH}}$	$\left[ \begin{array}{c} \text{CO}_2- \\ \diagup \\ \text{R} \\ \diagdown \\ (\text{NH}_2) \\ \diagdown \\ (\text{NH}_2) \end{array} \right]' \text{Na}^{\bullet}$
{ Lysine .....			
{ Arginine .....	$\begin{array}{c} \text{NH}_2 \\ \vdots \\ \text{CO}_2\text{H} \end{array} \text{R} - \text{NH}_2$	$\xrightarrow{+ 0. \text{NaOH}}$	$\begin{array}{c} \text{NH}_2 \\ \vdots \\ \text{CO}_2\text{H} \end{array} \text{R} - (\text{NH}_2)$
(4) {	$\begin{array}{c} \text{CO}_2\text{H} \\ \diagup \\ \text{R} \\ \diagdown \\ \text{CO}_2\text{H} \\ \diagup \\ \text{NH}_2 \cdot \text{HCl} \end{array}$	$\xrightarrow[\text{NaOH}]{\text{NaOH}}$	$\left[ \begin{array}{c} \text{CO}_2- \\ \diagup \\ \text{R} \\ \diagdown \\ \text{CO}_2- \\ \diagup \\ (\text{NH}_2) \end{array} \right]'' \text{Na}^{\bullet}$ $\text{Na}^{\bullet}$
(5) { Salts of amino acids	$\begin{array}{c} \text{CO}_2\text{H} \\ \diagup \\ \text{R} \\ \diagdown \\ \text{NH}_2 \cdot \text{HCl} \end{array}$	$\xrightarrow[\text{NaOH}]{+\text{NaOH}}$	$\left[ \begin{array}{c} \text{CO}_2- \\ \diagup \\ \text{R} \\ \diagdown \\ (\text{NH}_2) \end{array} \right]' \text{Na}^{\bullet}$ $+ \text{Na}^{\bullet} \text{Cl}'$
(6) {	$\begin{array}{c} \text{CO}_2\text{H} \\ \diagup \\ \text{R} \\ \diagdown \\ \text{NH}_2 \cdot \text{HCl} \\ \diagdown \\ \text{NH}_2 \cdot \text{HCl} \end{array}$	$\xrightarrow[\text{NaOH}]{+\text{NaOH}}$	$\left[ \begin{array}{c} \text{CO}_2- \\ \diagup \\ \text{R} \\ \diagdown \\ (\text{NH}_2) \\ \diagdown \\ (\text{NH}_2) \end{array} \right]' \text{Na}^{\bullet}$ $+ 2 \text{Na}^{\bullet} \text{Cl}'$

Notes.—The bracketed symbol (NH<sub>2</sub>) in this table denotes that the amino-group is in

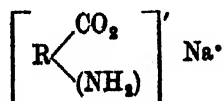
With a *monoamino-monocarboxylic-acid* in alcoholic solution neutralisation of the carboxyl group is complete at about P<sub>H</sub> 9.6, instead of at about 11.73 as in aqueous solution. The addition of an equivalent of soda will therefore result in the formation of the compound—

## XX.

by titration to thymolphthalein, and back titration to methyl-red.

Back titration with N/10 HCl to methyl-red.	Compounds shown below are not actually formed.	Body formed neutral to methyl-red.	Ratio th.ph. titration me. red titration
$\xrightarrow{+HCl}$	$\left( R \begin{array}{l} \text{CO}_2 \cdot \text{Na} \\ \text{NH}_2 \cdot \text{HCl} \end{array} \rightarrow \right)$	$R \begin{array}{l} \text{CO}_2\text{H} \\ \text{NH}_2 + \text{Na}^+ \text{Cl}' \end{array}$	1 : 1
$\xrightarrow{+HCl}$	$\left( R \begin{array}{l} \text{CO}_2\text{Na} \\ \text{CO}_2\text{Na} \\ \text{NH}_2 \cdot \text{HCl} \end{array} \rightarrow \right)$	$\left[ R \begin{array}{l} \text{CO}_2- \\ \text{CO}_2\text{H} \\ \text{NH}_2 \end{array} \right]' \text{Na}^+ + \text{Na}^+ \text{Cl}'$	2 : 1
$\xrightarrow{+2HCl}$	$\left( R \begin{array}{l} \text{CO}_2\text{Na} \\ \text{NH}_2 \cdot \text{HCl} \\ \text{NH}_2 \cdot \text{HCl} \end{array} \rightarrow \right)$	$\left[ R \begin{array}{l} \text{CO}_2\text{H} \\ \text{NH}_2- \\ \text{NH}_2 \end{array} \right] \cdot \text{Cl}' + \text{Na}^+ \text{Cl}'$	1 : 2
$\xrightarrow{+HCl}$	$\left( \begin{array}{l} \text{NH}_2 \\ \vdots \\ \text{CO}_2\text{H} \end{array} \right) R - \text{NH}_2 \cdot \text{HCl} \rightarrow \right)$	$\left[ \begin{array}{l} \text{NH}_2 \\ \vdots \\ \text{CO}_2\text{H} \end{array} \right) R - \text{NH}_2 - \right] \cdot \text{Cl}'$	0 : 1
$\xrightarrow{+HCl}$	$\left( R \begin{array}{l} \text{CO}_2\text{Na} \\ \text{CO}_2\text{Na} \\ \text{NH}_2 \cdot \text{HCl} \end{array} \rightarrow \right)$	$\left[ R \begin{array}{l} \text{CO}_2- \\ \text{CO}_2\text{H} \\ \text{NH}_2 \end{array} \right]' \text{Na}^+ + \text{Na}^+ \text{Cl}'$	3 : 1
$\xrightarrow{+HCl}$	$\left( R \begin{array}{l} \text{CO}_2\text{Na} \\ \text{NH}_2 \cdot \text{HCl} \end{array} \rightarrow \right)$	$R \begin{array}{l} \text{CO}_2\text{H} \\ \text{NH}_2 \end{array} + \text{Na}^+ \text{Cl}'$	2 : 1
$\xrightarrow{+HCl}$ $\xrightarrow{+HCl}$	$\left( R \begin{array}{l} \text{CO}_2\text{Na} \\ \text{NH}_2 \cdot \text{HCl} \\ \text{NH}_2 \cdot \text{HCl} \end{array} \rightarrow \right)$	$\left[ R \begin{array}{l} \text{CO}_2\text{H} \\ \text{NH}_2- \\ \text{NH}_2 \end{array} \right] \cdot \text{Cl}' + \text{Na}^+ \text{Cl}'$	3 : 2

the non-ionised form in alcoholic solution or as methylene derivative.

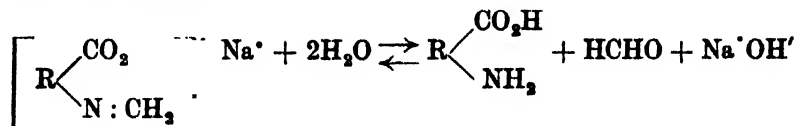


and the production of the coloured form of thymolphthalein. The symbol

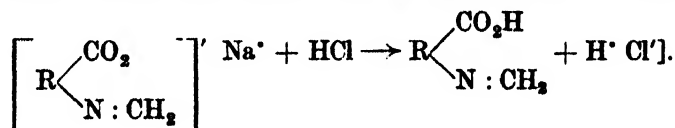
(NH<sub>2</sub>) indicates that the amino-group is in the non-ionised form in alcoholic solution (or as methylene derivative, if the solution contains formaldehyde).

At P<sub>H</sub> about 5.2 the amino-acid in alcoholic solution is non-ionised with respect to both amino- and carboxyl-groups, i.e., in the form of the undissociated acid. The addition of one equivalent of HCl to the sodium salt of a monoamino-monocarboxylic acid in alcoholic solution will therefore bring the mixture to the transition point of methyl-red.

[In the presence of formol the effect of increasing the C<sub>H</sub> to P<sub>H</sub> 5.2 ca. is also to send the reaction



from left to right, with the formation of the free amino-acid, which again is inappreciably dissociated between P<sub>H</sub> 4.71 and 6.60, as shown in fig. 2; and during the back titration this reaction occurs together with the following:—



With a *dicarboxylic-monoamino acid* in alcohol or formol solution the addition of two equivalents of soda is necessary in order to neutralise the two carboxyl-groups and produce a coloration of the thymolphthalein. At the transition point of methyl-red, however, one CO<sub>2</sub>H group is ionised as sodium salt, the remaining carboxyl- and amino-groups being uncombined. (The state of ionisation at P<sub>H</sub> 5.2 of the unmethylated amino-acid produced by the addition of acid to the sodium *methylene* amino-acid salt is shown in fig. 3 (p. 449 *supra*); one carboxyl group being dissociated.) Therefore in the back titration from thymolphthalein to methyl-red one equivalent of HCl is required to produce a body neutral to methyl-red. For the equation, see Table XX.

With lysine and arginine, one amino-group is ionised (as the mono-hydrochloride, etc.) in a solution neutral to methyl-red, the other groups are undissociated; lysine is neutralised to thymolphthalein as the sodium salt (with amino-groups undissociated), while arginine fails to show titratable carboxyl. Hence arginine titrates as though it were a simple monoamino base, the second amino- and the carboxyl-group failing to function.\* Lysine behaves normally as its formula indicates, as a diamino-monocarboxylic body.

\* A consequence of the low dissociation constants—see Parts 1 and 4.

The reaction which occurs when *salts* are titrated will be clear from reference to Table XX.

The values obtained in the estimations of *carboxyl* (plus other acidic groups) by titrating in alcohol to thymolphthalein, and of *amino* by back titration to methyl-red, are summarised in the following table. The quantities of the various amino-acids employed and the concentration of alcohol are the same as those indicated in Table XIX.

TABLE XXI.

	Estimation of —COOH and other acidic groups.		Estimation of —NH <sub>2</sub> .	
	c.c. of N/10 NaOH required titrating in alcohol to thymol-phthalein.		c.c. of N/10 HCl required in back titration to methyl-red.	
	Found (corr.).	Theoretical.	Found (corr.).	Theoretical.
1. Glycine .....	5.0	5.0	5.0	5.0
2. Alanine .....	4.95	5.0	4.95	5.0
3. Valine .....	2.4	2.5	2.4	2.5
4. Leucine .....	4.9	5.0	4.9	5.0
5. Cystine .....	5.05	5.0	4.9	5.0
6. Phenylalanine-hydrochloride .....	10.05	10.0	5.05	5.0
7. Tyrosine .....	6.4	5.0 to 10.0 (6.4 for 28 per cent. of phenolic dissn.)	6.3	5.0 to 10.0 (6.4 for 28 per cent. of phenolic dissn.)
8. Tryptophane .....	5.0	5.0	5.0	5.0
9. Aspartic acid .....	9.75	10.0	4.6	5.0
10. Glutaminic acid hydrochloride .....	5.9	6.0	1.95	2.0
11. Histidine hydrochloride, H <sub>2</sub> O .....	2.0	2.0	1.0	1.0
12. Lysine picrate .....	2.5	2.5	2.5	2.5
13. Arginine .....	0.05	0.0	4.4*	5.0
14. Mixture of glycine, leucine, valine, glutaminic—HCl .....	5.8	6.0	3.9	4.0

The end-points in all cases were sharp and well defined within one or two drops.

*Determination of Blank Correction.*

5 c.c. of water (CO<sub>2</sub> free) + 50 c.c. of alcohol.

Required, titrating to thymolphthalein..... 0.2 c.c. of N/10 NaOH

„ back-titrating to methyl-red ..... 0.25 c.c.

The results with phenolphthalein, and in presence of formaldehyde† are also

\* Impure sample of arginine, containing 88 per cent. as determined also by titration with HCl to methyl-red in aqueous solution.

† See p. 509.

given as they throw light on the ionisation and on the stability of the methylene derivatives of the amino acids :—

EXPERIMENTAL RESULTS :—TITRATIONS TO PHENOLPHTHALEIN ; AND IN PRESENCE OF FORMALDEHYDE.

	c.c. of N/10 NaOH required, titrating to phenolphthalein
<i>Blank Titrations—</i>	
50 c.c. of alcohol + 10 c.c. of formol* . . . .	0.2 to 0.3.
	c.c. of N/10 HCl required titrating to methyl-red
Back titration of last to methyl-red . . . . .	0.5
<i>Blank Titrations—</i>	
50 c.c. of alcohol	
+ 5 c.c. of N/10 HCl	
+ 10 c.c. of formol†	
Required . . . .	5.15 c.c. N/10 NaOH
	c.c. of N/10 NaOH required, titrating to phenolphthalein
Therefore, blank = . . . . .	0.15
<i>Blank Titrations—</i>	
	c.c. of N/10 HCl required titrating to methyl-red
Back titration of last to methyl-red . . . . .	0.5
etc.	etc.

1. ALANINE.

1. <i>Carboxyl estimated.</i>	c.c. of N/10 NaOH required titrating to phenolphthalein
(a) 5 c.c. of M/10 alanine + 25 c.c. of alcohol . . . . .	2.9 ca. (corrected for nor- mality of titrant and for blank).
(b) Added 5 c.c. of diluted neutralised HCHO . . . . .	4.74     „     „ 4.76     „     „
(c) Added 25 c.c. more alcohol . . . . .	4.90     „     „
[Ideal . . . . .	5.00]

\* Prepared by diluting 5 c.c. of 40 per cent. formol with 5 c.c. of water and neutralising to phenolphthalein immediately before use.

† Prepared by diluting 5 c.c. of 40 per cent. formol with 5 c.c. of water, and neutralising to phenolphthalein.

2. $-\text{NH}_2$ estimated.		c.c. of N/10 HCl required titrating to methyl-red
(d) Back titration of solution (c) .....	4.89 (corrected)	
[Ideal .....	5.00]	
3. Back Titration to Cresol-red.		c.c. of N/10 HCl required titrating to cresol-red
(e) Back titration of solution (c) .....	4.9	
4. Effect of Addition of Alcohol on Formol Titration.		c.c. of N/10 NaOH required titrating to phenolphthalein
(f) 5 c.c. of M/10 alanine .....	(1½ drops)	
(g) + 5 c.c. of prepared formol.....	3.8 c.c. (corrected for blank only).	
(h) Added 7 cc. of alcohol .....	4.68	„ „
(i) „ 12 „ in all .....	4.82	„ „
(j) „ 15 „ „ .....	4.86	„ „
(k) „ 25 „ „ .....	4.88	„ „
(l) „ 50 „ „ .....	4.97	„ „
(m) „ 100 „ „ .....	5.04	„ „
[Ideal.....	5.00]	

## 2. ASPARTIC ACID.

1. $\text{CO}_2\text{H}$ estimated.		c.c. of N/10 NaOH required titrating to phenolphthalein
(a) 5 c.c. of M/20 aspartic acid*		
+ 50 c.c. of alcohol.....	3.65	
(b) Added to (a), 10 c.c. prepared formol..	{ 5.0 4.9 }	(corr.)
[Ideal .....	5.0]	
2. $\text{NH}_2$ estimated.		c.c. of N/10 HCl required back titration to methyl-red
(c) Back titration of solution (b) .....	2.4 (corr.)	
[Ideal .....	2.5]	

\* Brought into solution at 100°.

3. *Effect of Increasing the Concentration of HCHO in Estimation of CO<sub>2</sub>H.*

	c.c of N/10 NaOH required titrating to phenolphthalein
(d) 5 c.c. of M/20 aspartic acid and 2.5 c.c. of prepared formol .....	4.5 (uncorr.)
(e) 5 c.c. of M/20 aspartic acid and 5 c.c. of prepared formol .....	4.75 „
(f) 5 c.c. of M/20 aspartic acid and 7 c.c. of prepared formol .....	4.85 „
(g) 5 c.c. of M/20 aspartic acid and 10 c.c. of prepared formol .....	5.0 „
(h) 5 c.c. of M/20 aspartic acid and 20 c.c. of prepared formol .....	5.15 „
[Ideal .....	5.0 (corr.)]

4. —NH<sub>2</sub> estimated.c.c. of N/10 HCl required in  
back titration to methyl-red

(i) Back titration of solution (h) .....	2.5 (corr.)
[Ideal .....	2.5 „ ]

5. —CO<sub>2</sub>H estimated in Aqueous Formol Solution.c.c. of N/10 NaOH required  
titrating to phenolphthalein

(j) 5 c.c. of M/20 aspartic acid + 5 c.c. formol .....	4.8
[Ideal .....	5.0]

6. —NH<sub>2</sub> estimated in Aqueous Formol Solution.c.c. of N/10 HCl required  
back titration to methyl-red

(k) Back titration of solution (j) .....	2.5 (corr.)
[Ideal .....	2.5]

## 3. GLUTAMINIC ACID HYDROCHLORIDE.

1. *Estimation of Carboxyl plus Hydrochloric Acid.*c.c. of N/10 NaOH required,  
titrating to phenolphthalein

(a) 5 c.c. of M/10 glutaminic acid — HCl + 50 c.c. of alcohol .....	
+ 10 c.c. of prepared HCHO ....	12.15

	c.c. of N/10 NaOH required titrating to phenolphthalein
(b) Addition of further 25 c.c. of alcohol to	
(a) making 75 c.c. in all . . . . .	14.8
[Ideal . . . . .	15.0]
2. <i>Estimation of NH<sub>2</sub>.</i>	c.c. of N/10 HCl required in back titration to methyl-red
(c) Back titration of solution (b) . . . . .	5.1 (corr.)
[Ideal . . . . .	5.0]
3. <i>Experiments with Aqueous Formol Solution.</i>	
(d) 5 c.c. M/10 glutaminic acid HCl . . . . .	Required
neutralised to ph.ph. . . . .	9.8 c.c. N/10 NaOH
(e) Added 5 c.c. of prepared HCHO to (d)	
neutralised to ph.ph. . . . .	12.7   ,,   ,,
[Ideal . . . . .	15.0]
(f) Added to 5 c.c. M/10 glutaminic acid — HCl + 10 c.c. prepared formol	
15 c.c. of N/10 NaOH (ideal quantity calculated)	
Required in back titration to methyl-	
red . . . . .	5.0 (corr.)
<i>The last result demonstrates the neutrality of the mono-sodium salt of glutaminic acid to methyl-red in the formol solution.</i>	
4. <i>Estimation of Carboxyl, using Thymol Blue* (acid range) in place of Phenolphthalein.</i>	
	c.c. of N/10 NaOH required
(g) 5 c.c. of M/10 glutaminic acid-HCl	
+ 10 c.c. of prepared formol	
+ 75 c.c. of alcohol . . . . .	14.8
[Ideal . . . . .	15.0]
5. —NH <sub>2</sub> estimated by Back Titration of Last.	c.c. of N/10 HCl required in back titration to methyl-red
(h) Back titration of (g) . . . . .	5.0 (corr.)
[Ideal . . . . .	5.0]

\* P<sub>H</sub> 8-9.0.



#### 4. GLUTAMINIC ACID HYDROCHLORIDE PLUS EXCESS OF FREE HCL.

1. $-\text{CO}_2\text{H}$ estimated.	c.c. of N/10 NaOH required titrating to phenolphthalein
2 c.c. of M/10 glutaminic acid-HCl	
+ 2 c.c. of N/10 HCl	
+ alcohol + formol .....	7.9
[Ideal .....	8.0]
2. $-\text{NH}_2$ estimated.	c.c. of N/10 NaOH required in back titration to methyl-red
Back titration of last .....	2.0
[Ideal .....	2.0]

#### 5. ARGININE DINITRATE.

1. <i>Titration in Water.</i>	c.c. of N/10 NaOH required titrating to phenolphthalein
(a) 5 c.c. of M/60 arginine dinitrate .....	0.8
[Ideal .....	0.83]
<i>This result illustrates the neutrality of arginine mononitrate in water.</i>	
2. <i>Titration in Aqueous Formol.</i>	
(b) 5 c.c. of M/60 arginine dinitrate	
+ 5 c.c. of neutralised HCHO ....	1.35
[Ideal .....	1.66]
3. <i>Titration in Alcohol.</i>	
(c) 5 c.c. of M/60 arginine dinitrate	
+ 50 c.c. of alcohol .....	$\begin{cases} 1.7 \\ 1.7 \end{cases}$
[Ideal .....	1.66]
4. <i>Titration in Aqueous Formol.</i>	
(d) Added 10 c.c. of neutralised HCHO to	
(a) .....	$\begin{cases} 1.8 \\ 1.75 \end{cases}$
[Ideal .....	1.66]

*The last three results show that the nitric acid titrates, but not the carboxyl group in arginine dinitrate.*

**5. Estimation of  $-\text{NH}_2$ .**

c.c. of N/10 HCl required in  
back titration to methyl-red

(e) Back titration of (c) .....	$\left\{ \begin{array}{l} 0.85 \\ 0.75 \end{array} \right\}$ (corr.)
[Ideal .....	0.83]

*Illustrating the neutrality of arginine mononitrate to methyl-red even in presence of alcohol.*

N.B.—One  $-\text{NH}_2$  exhibits no basic tendency.

**6. LYSINE PICRATE\* (IMPURE).**

**1. Estimation of Carboxyl Picric Acid.**

c.c. of N/10 NaOH required  
titrating to phenolphthalein

(a) 5 c.c. of M/40 lysine picrate + 40 c.c. of alcohol .....	$\left\{ \begin{array}{l} 2.35 \\ 2.55 \end{array} \right\}$ (corr.)
[Ideal .....	2.5]

**2. Estimation of  $-\text{NH}_2$ .**

c.c. of N/10 HCl required in  
back titration to methyl-red

(b) Back titration of solution (a) .....	$\left\{ \begin{array}{l} 2.2 \\ 2.3 \end{array} \right\}$
[Ideal .....	2.5]

**3. Effect of addition of Alcohol on Formol Titration.**

c.c. of N/10 NaOH required  
titrating to phenolphthalein

(c) 5 c.c. of M/10 lysine picrate .....	0.1
(d) 5 c.c. of M/10 lysine picrate + 10 c.c. of prepared formol .....	2.1 (corr.)
(e) + 10 c.c. of alcohol added to (d) .....	2.4 „
(f) + 20 c.c. of alcohol added to (d) .....	2.5 „
(g) + 50 c.c. of alcohol added to (d) .....	2.5 „
[Ideal .....	2.5]

\* Precise attainment of end-point in the following titrations was difficult owing to the yellow colour of the solution. Dissolved by putting containing vessel in hot water for a short time.

**7. LYSINE PICRATE, HYDROCHLORIC ACID MIXTURE.**

1. *Estimation of Total Acidic Groups.* c.c. of N/10 NaOH required  
titrating to phenolphthalein
- (a) 5 c.c. of M/40 lysine picrate  
+ 1.25 c.c. of N/10 HCl  
+ alcohol ..... 3.70  
[Ideal ..... 3.75]
2. *Estimation of  $-\text{NH}_2$ .* c.c. of N/10 HCl required in  
back titration to methyl-red
- (b) Back titration of solution (a) ..... 2.1 ca.  
[Ideal ..... 2.5]

**8. HISTIDINE MONOHYDROCHLORIDE.**

1. *Estimation of HCl.* c.c. of N/10 NaOH required  
titrating to phenolphthalein
- (a) 5 c.c. of M/20 histidine hydrochloride 2.55  
[Ideal ..... 2.5]
- Illustrating practical neutrality of histidine to phenolphthalein in aqueous solution.*
2. *Estimation of  $\text{CO}_2\text{H}$  and HCl.* c.c. of N/10 NaOH required  
titrating to phenolphthalein
- (b) 5 c.c. of M/20 histidine hydrochloride  
+ 50 c.c. of alcohol ..... 4.9  
[Ideal ..... 5.0]
3. *Estimation of  $\text{NH}_2$ .* c.c. of N/10 HCl required in  
back titration to methyl-red
- (c) Back titration of solution (b) ..... 2.45  
[Ideal ..... 2.5]
4. *Effect of Addition of Alcohol on Ionisation of  $-\text{NH}_2$ .* c.c. of N/10 NaOH required
- (d) 5 c.c. of histidine (free acid) + 50 c.c.  
of alcohol—  
Titrated to brom-phenol-blue  $\text{P}_H$   
colour 3.6 ..... 5
- (e) 5 c.c. of histidine aqueous solution.... 5

*The last results show that the addition of alcohol has little effect on the ionisation of the  $\text{NH}_2$  group, which is completely ionised as hydrochloride in the presence or absence of alcohol.*

### 9. ARGININE.

1. *Titration in Alcohol.* c.c. of N/10 NaOH required  
titrating to phenolphthalein

(a) 0.08709 grm. of arginine (impure)	
+ 2 c.c. of water	
+ 5 c.c. of alcohol . . . . .	0.05
[Ideal . . . . .	0.00]

N.B.—The carboxyl group in arginine exhibits no acidic properties even, in presence of alcohol.

2. *Estimation of one  $-\text{NH}_2$  Group.* c.c. of N/10 HCl required in  
back titration to methyl-red
- |  |           |
|--|-----------|
| Back titration of solution (a) . . . . . | 4.4 c.c.  |
| [Ideal for 100 per cent. purity          | 5.0 c.c.] |

N.B.—One of the two  $-\text{NH}_2$  groups exhibits no basic properties.

3. *Neutrality of Arginine Monohydrochloride to Methyl-red in Alcohol.*

Monohydrochloride of arginine prepared by titrating arginine solution in water with N/10 HCl to methyl-red. Same quantity of N/10 HCl was required in the presence of 80 per cent. alcohol.

N.B.—It is important to note, in view of the experimental investigations recorded above, that the methods advocated for estimating (1) carboxyl- and (2) amino-groups respectively in presence of alcohol are: (1) *titration to thymolphthalein*, (2) *back titration to methyl-red*. The results so obtained are summarised in Tables XIX and XXI above.

### SUMMARY.

#### *Part IV.*

The methods of Foreman and of Sørensen involving the titration of carboxyl after addition of alcohol or formaldehyde are discussed from the standpoint of the theory of titration, and it is concluded that the methods depend upon an increase (*ca.*  $\times 1,000$ ) in the value of  $K_a$  above that in aqueous solution.

#### *Part V.*

For conveniently estimating carboxyl or total acids, alcohol (80 per cent. of the total volume) and neutralised formol (5 per cent. of the total volume)

may be added to an amino-acid solution, which is then titrated with aqueous N/10 or N/1 NaOH to phenolphthalein, in slight modification of Foreman's method.

A preferable method consists in titrating with aqueous N/10 soda in the presence of 80 per cent. of alcohol to a blue colour with *thymolphthalein* (an indicator with a smaller  $K_a$  value). A very accurate estimation of carboxyl or total acidic groups is thereby obtained, including those amino-acids which give low readings with phenolphthalein; no formol need be added; and use of alcoholic potash is unnecessary. Under these conditions 24 per cent. of the feebly acidic phenolic group in tyrosine also titrates.

#### *Part VI.*

If to the solution thus neutralised to thymolphthalein, methyl-red be now added and standard HCl run in until the indicator assumes an orange colour, the amount of acid so required is approximately equivalent to the total amino-groups present. This result depends on the neutrality to methyl-red, even in presence of alcohol of the following bodies: Monoamino-monocarboxylic acids; monosodium salt of glutamic and aspartic acids; monohydrochlorides of arginine and lysine. The method is of use in distinguishing between the three types of amino-acids.

Blank corrections should be applied to the two titrations.

In arginine one of the two  $\text{NH}_2$  groups and the  $\text{CO}_2\text{H}$  both fail to titrate owing to low  $K_b$  and  $K_a$  values,

I should like to express my appreciation of the kind interest Professor F. G. Hopkins, F.R.S., has taken in this work, and to thank him and Mr. J. B. S. Haldane for kindly having read the manuscript. My thanks are due also to the Scientific and Industrial Research Department for a grant.

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#### ERRATUM IN PART II.

P. 482, line 4 from bottom, *for* earth *read* early.

## *Lunar Periodicity in Reproduction.*

By H. MUNRO FOX, Fellow of Gonville and Caius College, Cambridge.

(Communicated by Prof. J. S. Gardiner, F.R.S. Received June 15, 1923.)

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### 1. *Introduction.*

In Volume 2 of the 'Philosophical Transactions' of the Royal Society, published in 1667, travellers to the East Indies are asked to enquire (p. 419) "whether those shell-fishes, that are in these parts plump and in season at the full moon, and lean and out of season at the new, are found to have contrary constitutions in the East Indies?" This belief that the size of certain marine invertebrates, chiefly molluscs and echinoderms, varies with the phases of the moon is found in the literature of classical Greece and Rome and of the middle ages, and is held to-day in the fish markets around the Mediterranean and in the Red Sea. At Suez sea-urchins and crabs are said to be "full" at full moon and "empty" at new moon, at Alexandria the same thing is said of mussels and of sea-urchins, the Tarentines believe that oysters are fattest at full moon (34), while at Nice, Naples, Alexandria, and in Greece (17, p. 17, footnote) urchins are said to be fullest at full moon. The part of the sea-urchin which is eaten is the gonad, while in the crab it is the muscles, so that these tissues are supposed to vary in bulk with the phases of the moon. Now my own investigations, of which preliminary reports have already been published (8, 9) and a full account is to be given in this

paper, have shown that while the supposition is untrue of mussels (*Mytilus sp.*) and sea-urchins (*Strongylocentrotus lividus*) in the Mediterranean and of mussels (*Mytilus variabilis*) and crabs (*Neptunus pelagicus*) in the Red Sea, it is based on fact as concerns the sea-urchins found at Suez (*Centrechinus* [*Diadema*] *setosus*). In the last-mentioned form the gonads undergo a cycle of growth and development corresponding with each lunation throughout the breeding season. Just before full moon ovaries and testes are at their greatest bulk, filled with spermatozoa or eggs which are spawned into the sea at the time of full moon. The shrunken gonads then gradually fill again with ripening sexual products to be shed at the next full moon. It is remarkable, then, that a belief which was such common knowledge among the ancient inhabitants of Mediterranean countries that it was referred to by their poets and orators and one which is held to-day in the Mediterranean ports is indeed untrue of this region, while at Suez it is based on fact. It is possible that the Greeks originally obtained the belief from the ancient Egyptians, who would have it from the Red Sea (what really occurs there in sea-urchins being supposed to apply to all "shell-fish"), and that the same belief has survived around the Mediterranean until to-day. Or perhaps the belief had an independent origin in Greece, founded, not on fact, but on the supposed influence of the moon-deities on growth in general.

The principal classical references to this supposed lunar effect on "shell-fish" are the following :—

First among the Greek authors, Aristotle (*De Part. Anim.* IV, 5, ed. Didot, vol. 3, p. 280, 14ff.), discussing the fact that shell-fish cannot support extremes of temperature, says σημείον δὲ τὸ συμβαίνειν ἐπὶ τῶν ἐχίνων εἰδὴς τε γὰρ γινόμενοι ἔχουσι [sc: φά], καὶ ἐν τοῖς πανσελήνοις μᾶλλον. Antigonus Carystius ('*Hist. mir.*' 124) has καὶ τὰ τῇ σελήνῃ συναυξανόμενά τε καὶ συμφθίνοντα, ὅλον τὰ τῶν μῶν ἥπατα . . . καὶ τὰ τῶν θαλαττίων δὲ ἐχίνων φὰ ταὐτὸ πᾶσχειν κ. τ. λ. Oppian, in his poem on fishing, the '*Haliutica*,' writes (lib. V, v. 589, seq., ed. Rittershusius, *Lugd. Bat.*, 1697), "Genera verò ostracea, quæ serpunt (in) mari, omnia rumor (est) luna quidem augescante secundum orbem carne repleta esse, et maiorem habitare domum: decrescente verò rursum tenuioribus membris corrugari."

Lucilius repeats the same story (*Caii Lucillii Satyrarum*, ed. F. Dousa, *Lugd. Bat.*, 2nd ed. Patavii, 1735, fr. 30, p. 44): "Luna alit ostrea et implet echinos." Cicero (*De Div.*, lib. II, cap. 14, 33) says "(dicuntur) ostrisque et conchyliis omnibus contingere, ut cum luna pariter crescant pariterque decrescant." Horace in his *Satires* (IV, lib. II, 4, 30) writes "Lubrica

nascentes implent conchylia lunæ." Manilius has ('Astron,' II, 93) "Sic submersa fretis concharum et carcere clausa ad lunæ motum variant animalia corpus." Pliny makes several references to the phenomenon, of which the most definite are, first (Hist. nat., lib. II, cap. 41), "lunari potestate ostrearum conchiliorumque, et concharum omnium, corpora augeri ac rursus minui . . . exquisivere diligentiores"; and second (Hist. nat., lib. IX, cap. 31), "omnia ejus generis (cancri, echini) hieme læduntur, autumno et vere pinguescunt, et plenilunius magis." Finally, Aelian states ('De Natura Animalium,' lib. IX, cap. 6): τῶν δὲ ὀστρακονότων τε καὶ ὀστρακοδέρμων καὶ τοῦτο ἴδιον, κενώτερά πως ταῦτα καὶ κουφότερα ὑποληγοῦσης τῆς σελήνης φιλεῖ γίνεσθαι.

In the fifth century St. Augustine writes ('De Civitate Dei,' lib. V, cap. 6), " . . sicut in solaribus accessibus et decessibus videmus etiam ipsius anni tempora variari, et lunaribus incrementis atque decrementis augeri, et minui quædam genera rerum, sicut echinos et conchas . . ." Finally, Francis Bacon characteristically suggests an experimental investigation ('Sylva Sylvarum,' 1627, cent. IX, sect. 892): " . . . the opinion received is that . . . brains in rabbits, woodcocks, calves, etc., are fullest in the full of the moon . . . and so of oysters and cockles, which of all the rest are the easiest tried, if you have them in pits."

## 2. Lunar Periodicity in the Reproduction of a Red Sea Echinoid (*Centrechinus Setosus*).

To test the truth of the popular belief regarding sea-urchins at Suez, I made systematic examinations of the gonads of *Centrechinus (Diadema) setosus* at this place in July, August and September, 1920 and 1921. The material was obtained with a hand-net along the sides of the eastern stone jetty at the entrance to the Docks of Port Taufiq. The microscopic examinations of the living gonads were made in the Quarantine Laboratory. The result confirmed the reports of the fish-vendors to a surprising degree. There is a periodic reproductive cycle correlated with the lunar month, the genital products being spawned round about each full moon until the breeding season closes in September, after each spawning period the testes and ovaries being somewhat reduced in bulk. During the declining phases of the moon a fresh crop of genital products is being formed. As the time of new moon is passed these forming germ-cells advance in development, and concomitantly the visible size of the genital glands increases slightly once more, until, about the time of full moon, spermatozoa or eggs are again shed.



Table I.—State of Maturity of *Centrechinus setosus* at Suez, July-September, 1920.

Date.	Percentages of males which spawned on laboratory bench.	Numbers of males examined.	Percentages of females in which the ovaries contained ripe eggs only.	Numbers of females examined.
15.7.20			0	5
21.7.20			83	6
29.7.20			24	4
6.8.20			0	5
9.8.20			0	8
12.8.20	0	5	0	7
16.8.20	0	7	17	6
18.8.20	71	7	20	5
20.8.20	74	11	20	5
22.8.20	50	6	37	8
24.8.20	43	7	14	7
27.8.20	10	10	36	11
30.8.20	0	8	12	8
3.9.20	10	10	0	7
5.9.20	0	11	0	8
8.9.20	0	9	0	7
11.9.20	0	7	0	7
13.9.20	0	8	0	7
15.9.20	0	9	0	7
17.9.20	0	9	0	8
20.9.20	0	8	0	7
24.9.20	0	10	0	8
28.9.20	0	9	0	7

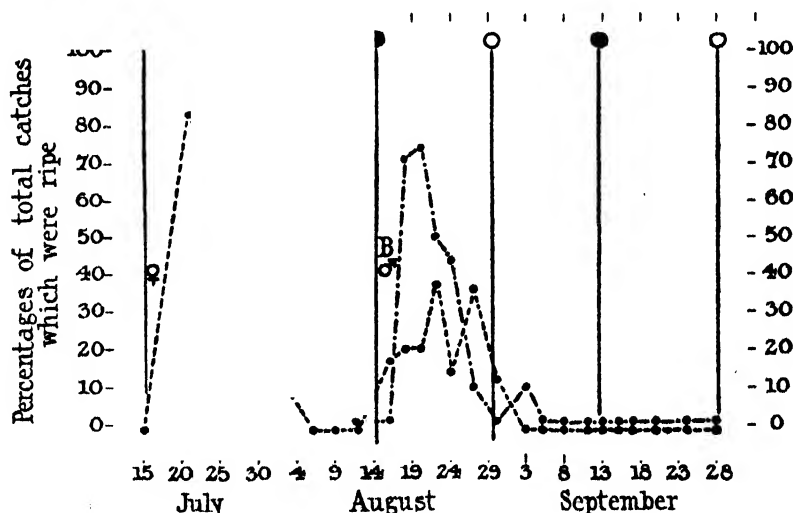


FIG. 1.—State of maturity of *Centrechinus setosus* at Suez in 1920. Curve ♂ B shows the percentages of males which spawned on the laboratory bench and curve ♀ gives the percentages of females in which the ovaries contained ripe eggs only. The dates of full and of new moon are indicated by vertical lines with respectively white and black circles.

Table II.—State of Maturity of *Centrechinus setosus* at Suez, July-September, 1921.

Date.	Percentages of males in which the teased testes showed spermatozoa only, without admixture of spermatocytes.	Percentages of males which spawned on the laboratory bench.	Numbers of males examined.	Percentages of females in which the ovaries contained ripe eggs only.	Numbers of females examined.
3.7.21 .....	73	40	15	10	19
10.7.21 .....	83	58	24	39	13
17.7.21 .....	100	65	17	85	13
25.7.21 .....	31	10	19	9	21
1.8.21 .....	0	0	11	0	16
7.8.21 .....	22	19	27	0	9
15.8.21 .....	78	53	18	56	16
23.8.21 .....	68	18	22	30	20
31.8.21 .....	0	0	19	0	20
8.9.21 .....	14	0	21	7	15
16.9.21 .....	24	5	21	0	17
21.9.21 .....	7	0	15	5	22
28.9.21 .....	0	0	13	0	11

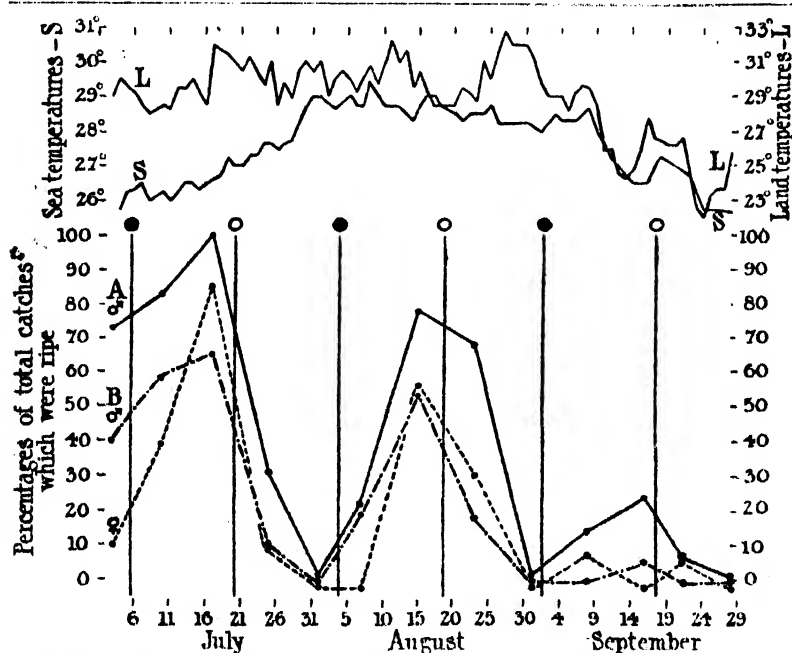


FIG. 2.—State of maturity of *Centrechinus setosus* at Suez in 1921. Curve ♂ A shows the percentages of males in which the teased testes gave spermatozoa only, without admixture of spermatocytes; ♂ B shows the percentages of males which spawned on the laboratory bench; and ♀ gives the percentages of females in which the ovaries contained ripe eggs only. The dates of full and of new moon are indicated by vertical lines with respectively white and black circles. The daily sea-temperatures and the average daily land-temperatures are entered above.

The numerical results of the microscopic examinations of the gonads are given in Tables I and II, and in figs. 1 and 2. The numbers of each sex examined are noted in the Tables. The average size of the urchins used was always the same. The 1921 data are better than on those of 1920, since in 1921 a greater number of individuals was investigated on each occasion. The correspondence of the curves for the two years is satisfactory.

The data represent the percentages of the totals of each sex examined in which (1), in the case of males, a piece of testis teased in a drop of water on a slide showed spermatozoa alone, without admixture of spermatocytes, or (2), in the case of females, ripe eggs alone, without any unripe eggs or oocytes, came from the teased ovary. These were the criteria of ripeness used.

The 1921 curves show (1) an exact correspondence between the ♂ curve and the ♀ curve—they rise and fall together, (2) the 3 apices of the curves occur between the 1st quarter and full moon, (3) the 3 points at which the curves touch the zero ordinate occur between the last quarter and new moon, (4) on each successive occasion the maxima are less high, i.e., progressively fewer individuals reach sexual maturity as the breeding season approaches its end.

Between the 1st quarter and full moon, when the maximum numbers of ripe individuals are found, there are present, in addition to those in which the teased gonads show nothing but spermatozoa or ripe eggs, others with numerous spermatozoa and ripe eggs, and in addition some spermatocytes or unripe eggs and oocytes, i.e., individuals not yet fully ripe. Further, there are some individuals with nothing but spermatocytes or small oocytes. These will not reach sexual maturity during the lunar period in question. In July this last type was non-existent in the ripe males, 100 per cent. of which became ripe, and it was infrequent in the females. In August it was more frequent, while in September when the breeding season was nearly over it formed the greater proportion of the totals.

Between full moon and the 3rd quarter, the gonads in a few individuals contain nothing but spermatozoa or ripe eggs, these few not yet having spawned. The majority, however, are either "spent," that is the gonads contain a very few spermatozoa or ripe eggs, which failed to be extruded at spawning, together with numerous spermatocytes and small oocytes, or they have gonads containing nothing but spermatocytes and small oocytes.

Between the 3rd quarter and new moon, at the close of the July, August and September periods, no urchins were found with gonads full of spermatozoa or eggs, but at the end of the June lunation, that is to say longer from the

end of the breeding season, it will be seen that the curves do not descend to the zero ordinate.

Between new moon and the 1st quarter some individuals are ripe, but not so many as after the moon's 1st quarter, while others are approaching ripeness, the gonads containing both spermatocytes and spermatozoa or both large oocytes and unripe eggs.

Fig. 1 shows 1920. The observations on the females were made exactly as in 1921, but the males were not examined microscopically. Instead, they were placed aboral pole downwards on the bench, and the numbers were noted in which spermatozoa exuded from the genital orifices after this treatment. Curve ♂ B gives the percentages of the totals examined in which spermatozoa exuded. To make the 1920 ♂ data comparable with those of 1921, in the latter year all urchins were put upside down on the bench before being cut open for microscopic examination and the proportion of males in which spermatozoa exuded was again noted. This is represented by Curve ♂ B of fig. 2. It will be seen that in this figure Curves ♂ B and ♂ A correspond, so that this method gives as true a measure of the spermatozoa-content of the testes as does microscopic examination.

The 1920 and 1921 curves are similar. The double apex of Curve ♀ during the August 1920 lunation I ascribe to the paucity of the numbers examined.

Both living material and sections of the gonads show a lunar cycle for the "cellules vésiculeuses" of Caullery (4).

No individuals reached sexual maturity in September 1920. In 1920 the September full moon fell on the 28th, whereas in 1921 it was on the 17th. Evidently in 1920 the September lunation fell too late to be included in the breeding season, which accordingly closed at the end of August. The progressive diminution of the numbers of individuals reaching sexual maturity in the later lunations, and the final end of the breeding season, is probably due to internal changes of the nature of fatigue, for there is no correspondence with any external change such as temperature. The sea-temperatures, taken at 11 a.m. daily close to the urchin beds, are entered in fig. 2. The temperatures during the 2nd quarter of the July moon are about the same as those in the 2nd quarter of the September moon, yet in the former period 100 per cent. of males and 65 per cent. of females were ripe while in the latter are only 24 per cent. males and 0 per cent. females were ripe. In the August lunar period the temperature was higher than in either July or September, but the degree of ripeness of the urchins was intermediate. Orton (32) has shown that the European oyster, wherever be its habitat, begins to spawn at 15°-16°, and

continues to produce sexual products as long as the temperature remains above this figure. This is not the case with *Centrechinus*, for its breeding season begins at Suez some months previous to July—I have not had the opportunity of fixing the exact date—at a temperature well below that of July and September, yet from July onwards, with the temperature still rising, the numbers of individuals reaching maturity declines, and in September all breeding ceases although the temperature is still above that at which the breeding season was initiated.

To return to the lunar periodicity, it would appear from the curves that one and the same individual may spawn and re-attain sexual maturity in consecutive lunar periods. In the 2nd quarter of the July 1921 moon 100 per cent. males were ripe and all of these spawned, for in the 4th quarter no ripe males were found. In the 2nd quarter of the August moon 78 per cent. of the males taken were ripe and all of these mature individuals must have spawned a month before and re-formed their spermatozoa since.\*

Such a rapid development of echinoid germ-cells is not without a parallel among the few cases in which the rate of growth is known. Loeb (25, p. 274) found that ripe eggs of *Strongylocentrotus purpuratus* in California were re-formed 10 days after spawning. The sea-temperature was about 12°–15°. Tennent, writing of Tortugas, says (38, p. 657, footnote): "During the three summers preceding 1908 and again in 1908, I noticed that the gonads of sea-urchins taken after a night of full moonlight were empty, while those obtained a week later gave abundance of eggs and spermatozoa."† Otto Koehler (19, p. 256) showed that in the Naples Aquarium tanks during the summer *Strongylocentrotus lividus* takes 1½–2 months to form ripe genital products.

### 3. Reproduction of Other Echinoids.

The echinoids (*Strongylocentrotus* [*Paracentrotus*] *lividus*) at Alexandria were next examined. The material was purchased in the Cairo fish-market, where the sea-urchins are exposed for sale on the day after that on which they are caught at Alexandria. They are obtained in 2–6 metres of water along the harbour breakwater. The germ-cells of urchins bought in Cairo are in good condition, since when ripe fertilisation can readily be effected. The examinations were made in the Biological Department of the School of Medicine, Cairo,

\* The only alternative explanation would be that after spawning the urchins retire into deep water and remain there, while a fresh lot with developing gonads migrate up to the coast to spawn at the next full moon. There is no evidence for this.

† This suggests a lunar reproductive periodicity similar to that of *Centrechinus setosus*, but the case requires further investigation.

Table III.—State of Maturity of *Strongylocentrotus lividus* at Alexandria, March–June, 1921.

Date of fishing.	Percentages of males in which the teased testes showed spermatozoa only, without admixture of spermatocytes.	Numbers of males examined.	Percentages of females in which the ovaries contained ripe eggs only.	Numbers of females examined.
3.21 .....			0	11
3.21 .....			9	11
3.21 .....			28	14
4.21 .....	18	16	0	10
4.21 .....	66	9	7	15
4.21 .....	63	8	0	12
4.21 .....	66	12	27	11
4.21 .....	25	12	8	12
4.21 .....	56	15	11	9
4.21 .....	45	11	16	13
5.21 .....	15	20	14	14
5.21 .....	38	21	14	15
5.21 .....	63	21	8	13
5.21 .....	55	21	21	15
5.21 .....	35	20	6	16
5.21 .....	75	17	19	20
5.21 .....	30	20	14	15
5.21 .....	17	18	26	19
5.21 .....	5	19	6	18
5.21 .....	0	14	8	13
5.21 .....	6	17	0	19

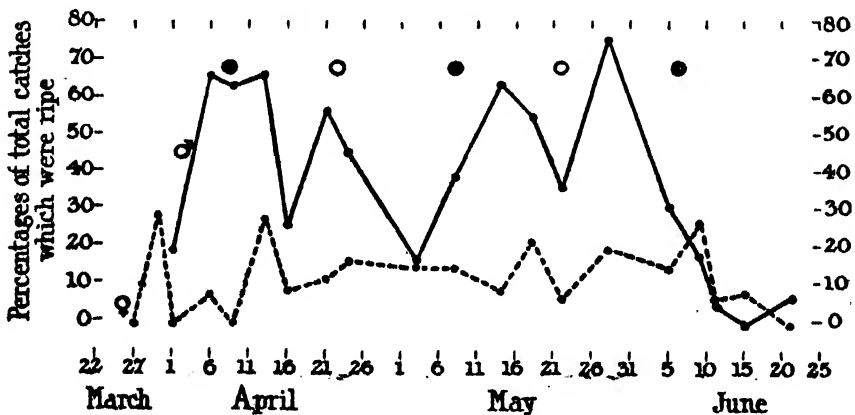


FIG. 3.—State of maturity of *Strongylocentrotus lividus* at Alexandria in 1921. Curve ♂ shows the percentages of males in which the teased testes gave spermatozoa only, without admixture of spermatocytes; curve ♀ shows the percentages of females in which the ovaries contained ripe eggs only. The dates of full and new moon are indicated by white and black circles respectively.

Table IV.—State of Maturity of *Strongylocentrotus lividus* at Alexandria, April–June, 1922.

Date of fishing.	Percentages of males in which the teased testes showed spermatozoa only, without admixture of spermatocytes.	Numbers of males examined.	Percentages of females in which the ovaries contained ripe eggs only	Numbers of females examined.
7.4.22	12	26	0	11
11.4.22	33	18	5	20
21.4.22	27	15	0	20
24.4.22	55	22	36	14
29.4.22	14	21	0	13
8.5.22	43	21	27	15
11.5.22	33	18	6	17
15.5.22	39	18	12	17
17.5.22	41	22	14	14
19.5.22	28	18	11	20
25.5.22	50	20	41	17
31.5.22	31	16	0	18
2.6.22	21	19	0	17
5.6.22	6	18	0	17
9.6.22	0	18	0	18
12.6.22	31	18	0	18

the observations extending over a little more than two lunar months during April to June, 1921 and 1922. Figures 3 and 4 show the results. There is no obvious lunar rhythm such as there was in *Centrechinus*, nor was any slighter and therefore not so apparent periodicity detectable by combining the data of the different lunations and plotting them according to days or groups of days after new moon. This negative result surprised me, since the belief in an influence of the moon is as strongly held by the people about this form as about the Red Sea urchin.

The most striking feature of the 1922 curves (fig. 4) is that there are considerable up and down fluctuations and that the maxima and minima correspond for the two sexes. On one day many males and females have their gonads filled with ripe germ-cells, on another day few individuals are in this condition. It appears that both sexes develop their sexual cells and then spawn simultaneously, but this spawning, unlike that of *Centrechinus*, occurs at irregularly spaced intervals. The 1921 curves (fig. 3) show, however, little correspondence between male and female fluctuations.

*Strongylocentrotus lividus* occurs all around the Mediterranean. The possibility of a lunar periodicity in its reproduction has also been investigated

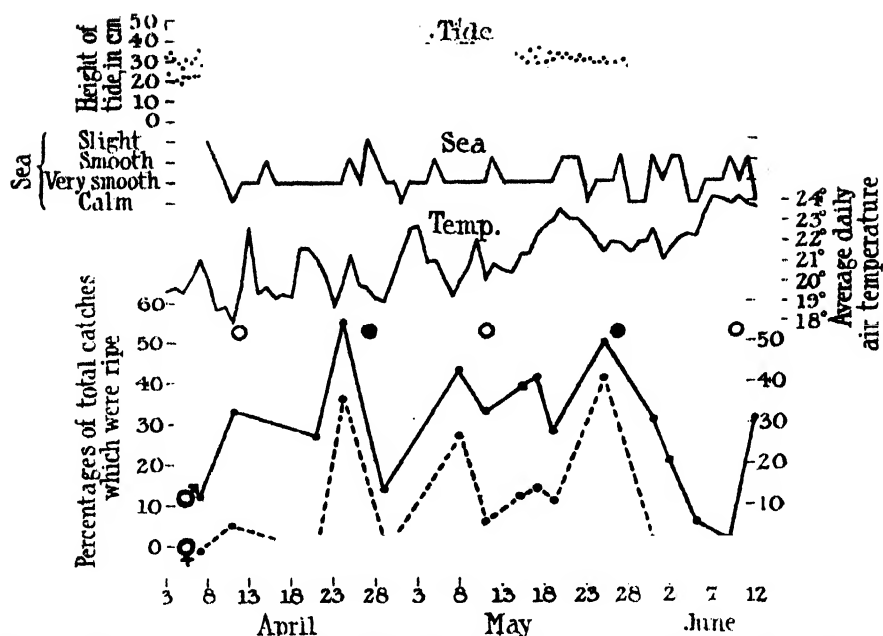


FIG. 4.—State of maturity of *Strongylocentrotus lividus* at Alexandria in 1922. Curve ♂ shows the percentages of males in which the teased testes gave spermatozoa only, without admixture of spermatocytes; curve ♀ shows the percentages of females in which the ovaries contained ripe eggs only. The dates of full and new moon are indicated by white and black circles respectively. Above are drawn the curves of average daily air temperatures and of the state of the sea. At the top of the diagram the heights of high and low water are entered as given by the Alexandria harbour tide-gauge.

by Otto Koehler at Naples (19, p. 258). Every five days during a period of two months, from November to February, this worker examined 20 animals, without finding any regular variation in gonad size or contents. I have further studied the question with fixed material from Marseilles. On six occasions during the months of October to January a piece of gonad from each of 12 to 16 individuals of *Strongylocentrotus lividus* was fixed in strong Flemming, and subsequently sectioned. The ovary sections were divided into the following five classes :—

Class 1.—Oogonia and oocytes of various sizes.

Class 2.—Ditto, plus some ripe eggs.

Class 3.—Lumen full of ripe eggs.

Class 4.—A few small oogonia at walls, many disintegrating oocytes and eggs, and a few ripe eggs in lumen ("spent").

Class 5.—As 4, but without eggs in lumen.



These are apparently five successive stages in the development of the ovary, 1 and 2 previous to maturity, 3 ready to spawn, 4 and 5 subsequent to spawning. Table 5 gives the class-distribution of urchins on each occasion. Three of the dates occurred approximately at full moon, namely, 16.10.21, 11.12.21, 8.1.22; and three at about new moon, viz., 31.10.21, 27.11.21, 25.12.21. The Table shows no suggestion of a lunar periodicity. We are apparently dealing with an interim period between the end and the commencement of the breeding

Table V.—State of Maturity of Female *Strongylocentrotus lividus* at Marseilles, October 1921 to January 1922. The Table shows the numbers of individuals which, on the dates indicated, fell in the various classes.

Date.	16.10.21.	31.10.21.	27.11.21.	11.12.21.	25.12.21.	8.1.22
Number of days after new moon .....	15	0	27	12	26	11
Class 1 .....		2	5	2	1	1
Class 2 .....		1	2	5	1	1
Class 3 .....					4	4
Class 4 .....	2	1			1	
Class 5 .....	5	3				

season. On both dates in October the majority of individuals came in Class 5, but on the second occasion the next most frequent class was No. 1. On 27.11.21 most were in Class 1, on 11.12.21 in Class 2, and on 25.12.21 and 8.1.22 in Class 3. That is to say, in October we find at first mainly "spent" females and after that an increasing number with early stages of developing oocytes. This development continues progressively through November and December, until at Christmas and in January most of the individuals are ripe. Exactly comparable results were obtained from the males.

Thus *Strongylocentrotus lividus* has no lunar reproductive periodicity, a fact which, as pointed out in the Introduction, makes the ancient and modern beliefs in Mediterranean countries very remarkable.

At Plymouth I have worked with the eggs and spermatozoa of the several species of *Echinus* occurring there over a period of several years. Although not studied with a view to discovering any periodicity in the reproduction, yet had there been any such regular and marked rhythm as there is at Suez this would necessarily have been noticed, for in the Red Sea it is difficult in the height of the breeding season, or impossible towards its end, to obtain any ripe spermatozoa or eggs at the time of new moon. If, then, there is any lunar reproductive periodicity in *Echinus* at Plymouth it is little marked.

It will, I hope, be possible to discover from an examination of plankton from different parts of the world, which are the echinoids (or other marine animals with pelagic larvæ) that show a lunar periodicity in reproduction. For the periodic spawning of *Centrechinus*, for example, must be reflected in the plankton of the Gulf of Suez. The plutei must vary in numbers and in stage of development with the phases of the moon. Unfortunately, suitable plankton data do not seem to exist for tropical seas. Either samples have been taken from moving stations or from one place at intervals of time too far apart to show a lunar periodicity, if such existed. Collections are, however, now being made with this end in view.

#### 4. Possible Causes of Reproductive Periodicity in Echinoids.

An external rhythmic change in the environment with the lunar period might cause the reproductive periodicity of the echinoids in one of two ways. Either the periodic agent might act upon the developing germ-cells from the beginning of their growth, causing them to become ripe and ready for spawning at the time when the moon is full. Or, at the time of full moon, it might have a trigger-like action on the gonads already filled with genital products, causing these to be spawned. When empty the testes or ovaries would re-develop germ-cells to remain when ripe in the gonads until again released by the periodic trigger.\* But not only are we ignorant of the nature of the periodic external factor, but the causes of spawning in echinoids, periodic or non-periodic, are unknown.

The alternating spring and neap tides induce a reproductive rhythm in certain shore animals (Bohn, 3, Keeble, 18, *Convoluta*); *Amphitrite*, a polychæte, lays its eggs at spring tides at Woods Hole (Scott, 35). But whereas *Centrechinus* has a single reproductive cycle in each lunation there are two spring- and two neap-tidal periods, i.e., a double cycle. Nevertheless, during the summer months at Suez the new-moon spring tides have a greater range than the full-moon springs, so that the maximum tidal range is attained once only during each lunar month. The higher and lower water at the new-moon springs might react on the echinoids by the different hydrostatic pressure (affecting, e.g., the tension of dissolved gases) or by causing the animals to be at a greater or lesser distance than usual from the source of oxygen or of light. But the average excess tidal range at new-moon springs over that at full-moon springs

\* The first of these two alternatives applies to the Palolo worm (p. 543 below), for Mayer (28, p. 109) states that 12 hours before swarming time the eggs are not yet ripe.

during the period studied was only 57 cms.\* This small difference could scarcely affect the urchins, for they are not sessile animals but move actively and their vertical range of migration during the course even of an hour is often far in excess of this figure.

The possibility of tidal influence could be tested by keeping urchins in a floating cage. If the lunar reproductive cycle were thereby abolished the tidal connexion would be demonstrated; but a contrary result would not dispose of a possible influence of the tides, for an established rhythm in a physiological process is often persistent after the original cause has ceased to act. *Convoluta* reared in the laboratory keeps the habit acquired in the sea of laying its eggs at neap tides (Keeble 18). In any case the experiment of eliminating the tides was, unfortunately, impracticable with *Centrechinus* owing to its size. Fully-grown specimens measure over one foot from tip to tip of the spines, and it was impossible to obtain large enough floating boxes to contain the hundred or more individuals necessary.

The absence of a lunar periodicity at Alexandria, where the tides are small (the greatest tidal range over the period studied was 30 cms., and the least 1 cm.), would argue in favour of a tidal cause, but for the absence of a lunar rhythm in *Echinus miliaris* which lives between the tide marks at Plymouth where the tidal range is great, having (during the breeding season) a maximum value of 5.2 metres and a minimum of 3.7 metres. However, the genera of echinoids at Suez, Alexandria and Plymouth are not the same.

The moon might affect the echinoids through its light although this is identical with sunlight in nature, differing only in intensity. Their spectra do not differ and each is polarised in the same proportion, the polarised light being in each case a part of that which is reflected from the sky. No polarisation of the light received directly from the face of the moon can be detected with a Savart's prism. The additional illumination on certain nights of the month, however, over and above that received by the animals on every day alike, might although weak cause in them a longer or a shorter period of activity, e.g., for feeding, on those occasions. Extensive observations were made at Suez to test this. The amount of locomotion of urchins was observed on dark nights and on moonlight nights, but no difference was found between the two.

The possibility of a direct effect of the light of the moon on the echinoids could be tested by keeping specimens (1) in the dark and (2) exposed to continuous illumination, but again the large size of *Centrechinus setosus* made

\* This was determined from the Suez Canal Company's tide gauge at Port Taufiq. The average spring-tidal range at new moon was 196 cms., and at full moon 139 cms.

the experiment impossible at Suez. If moonlight has an effect this must necessarily be more constant in the cloudless summer nights of Egypt than in Europe. At Alexandria, where there is no lunar periodicity, the urchins are at a depth of 2 to 6 metres, whereas at Suez they are immediately below the surface and thus exposed to the light.

The source of the large amount of material necessary for the re-development of the genital products each lunar month must presumably come from without, for there is no other tissue in an echinoid comparable in bulk with the gonads from the wastage of which the latter might be formed, as in the case of the salmon's ovaries, which are developed at the expense of the muscles, etc. This suggests that the sea-urchins may feed more at certain phases of the moon than at others. However, a systematic examination of the gut-contents of specimens taken at dawn, (1) at full moon, and (2) at new moon, showed no difference either in quantity or nature of food.

There may finally be a periodic change in the chemical constitution of the sea-water correlated with the lunar month, due perhaps to undetected periodicities in currents, which would react on the echinoids. Such a variation might be detectable by suitable chemical analyses, but I had no equipment for making such and had to be content with taking a daily observation of the density of the sea-water. This fluctuated between 1029.4 and 1027.4, but showed irregular non-periodic variations only.

Coming now to the possible causes of the simultaneous though non-periodic spawning of the Alexandria urchins (*Strongylocentrotus lividus*), there is again the difficulty that nothing is known of the causes of the discharge of the genital products in echinoids. The tides at Alexandria, as mentioned above, although small in range are sensible. To show that there is in fact no relationship, between the tidal range and the variations in maturity of the urchins, the heights of high and low water (from the tide gauge in Alexandria Harbour) are entered in fig. 4.

Temperature has such a general relationship to the maturity of marine organisms (Orton 32) that an influence on the spawning of the Alexandria urchins suggests itself. Unfortunately, no sea-temperature records exist for Alexandria, only air-temperatures being available. The relationships between air and sea-temperatures are seen from fig. 2, where both are plotted for Suez. Although there is some correlation between the two it is not very marked. The Alexandria air-temperatures are plotted in fig. 3, and there is no obvious relationship between them and the maturity of the urchins.

Roughness of the surface of the sea might affect the urchins, either by causing

a movement of the deeper water (the urchins are found at 2 to 6 metres) or by obscuring the light. The state of the sea is plotted in fig. 4, but again there is no correlation.

Cloudiness of the sky cannot be a cause, for in the months of April to June the sky is almost uniformly cloudless.

No records exist of the salinity of the water, but this cannot be supposed to fluctuate, since from April 15th onwards there is no outflow of fresh-water in the neighbourhood of Alexandria.

There remain as a possible cause only hypothetical variations in the constitution of the water due to varying in-shore currents. At present there is no evidence either for or against this suggestion.

#### 5. Other Marine Invertebrates Examined for a Lunar Periodicity with Negative Results.

(a) *Molluscs*.—Mussels (*Mytilus* sp.) are eaten at Alexandria but the species occurring at Suez (*Mytilus variabilis*) is too small. In Alexandria the popular belief in a change in the bulk of the "flesh" of mussels correlated with the moon's phases is as strongly held as it is of the sea-urchins. Since variations in bulk in lamellibranchs depend chiefly on the state of development of the gonad, a systematic microscopic examination of the gonads of living specimens was made both of *Mytilus variabilis* at Suez and of *Mytilus* sp. from Alexandria. The material at Suez was obtained between the tide-marks at Port Taufiq, all specimens examined being taken from the same square foot of rock. *Mytilus* sp. from Alexandria was purchased in the Cairo fish-market. The result was to show no trace of a lunar periodicity in either form, but some evidence was obtained, both from Suez and Alexandria, of the same type of simultaneous non-periodic spawning which was found for *Strongylocentrotus lividus* at Alexandria. The curves showing the proportions of males and females having gonads filled with ripe spermatozoa or ova rise and fall together.\*

The details are given in Tables VI, VII and VIII, and in figs. 5, 6 and 7. The sea-temperatures at Suez are plotted in fig. 5, but show no relationship to the rise and fall of the ripeness curves of the mussels. This is unlike the case of the oyster. T. C. Nelson (31, fig. 2) has shown that rises and falls in water-temperature are exactly followed by rises and falls in the curve of frequency of oyster larvæ present in the water.

\* It is remarkable that a large proportion of the eggs in the ovaries were at all times found to be cytolized, even when, as at Suez, the animals were examined immediately after removal from the sea.

Table VI.—State of Maturity of *Mytilus variabilis* at Suez, July-September, 1921.

Date.	Percentages of males in which the teased testes gave spermatozoa only without admixture of spermatocytes.	Numbers of males examined.	Percentages of females in which the ovaries contained numerous eggs.	Numbers of females examined.
6.7.21	29	17	30	23
13.7.21	47	17	60	25
21.7.21	29	17	17	24
29.7.21	48	21	70	20
2.8.21	12	25	65	17
5.8.21	62	24	76	17
6.8.21	55	22	63	19
8.8.21	39	18	45	22
10.8.21	40	20	64	22
12.8.21	50	22	65	20
14.8.21	53	21	30	21
17.8.21	75	24	76	17
20.8.21	44	25	53	17
23.8.21	47	19	66	26
26.8.21	67	21	67	15
29.8.21	57	21	53	15
2.9.21	69	16	53	19

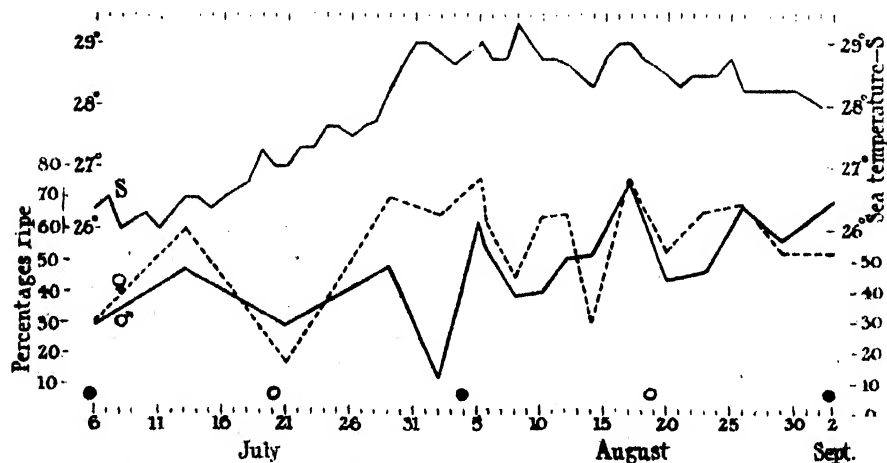


FIG. 5.—State of maturity of *Mytilus variabilis* at Suez, July-September, 1921. Curve ♂ gives the percentage of males in which the teased testis showed spermatozoa only, without admixture of spermatocytes; curve ♀ gives the percentage of females in which the ovary contained numerous eggs. The dates of full and new moon are marked by white and black circles respectively. The sea-temperatures are given by the top curve S.

Table VII.—State of Maturity of *Mytilus* sp. at Alexandria, May-June, 1921.

Date of fishing.	Percentages of males in which the teased testes gave spermatozoa only, without admixture of spermatocytes.	Numbers of males examined.	Percentages of females in which the ovaries contained numerous eggs.	Numbers of females examined.
30.5.21 .....	50	14	50	10
2.6.21 .....	66	12	62.5	13
6.6.21 .....	84	12	75	13
15.6.21 .....	50	10	50	16
22.6.21 .....	0	12	0	12

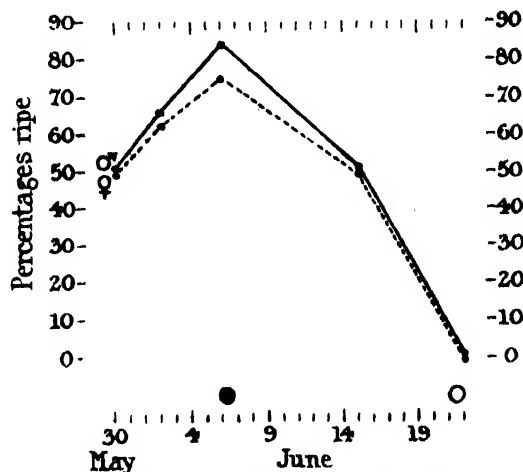


FIG. 6.—State of maturity of *Mytilus* sp. at Alexandria, May-June, 1921. Curve ♂ gives the percentage of males in which the teased testis showed spermatozoa only, without admixture of spermatocytes; curve ♀ gives the percentage of females in which the ovary contained numerous eggs. The dates of full and new moon are marked by white and black circles respectively.

It was mentioned in the Introduction that from ancient times to the present day oysters have been supposed to vary in bulk with the phases of the moon. The negative result of the *Mytilus* investigation made it very probable that this belief, too, was false, but it is not legitimate to generalise, since a lunar periodicity has lately been demonstrated in a mollusc. Crozier (6, p. 479, footnote) and Grave (10) have described the spawning of a chiton, *Chiton pleura apiculata*, at Woods Hole as taking place in the 3rd quarter of the moon.

Table VIII.—State of Maturity of *Mytilus* sp. at Alexandria, January-February, 1922.

Date of fishing.	Percentages of males in which the teased testes gave spermatozoa only, without admixture of spermatocytes.	Numbers of males examined.	Percentages of females in which the ovary contained numerous eggs.	Numbers of females examined.
3.1.22	81	16	100	26
12.1.22	66	21	100	15
17.1.22	100	25	100	11
24.1.22	38	16	89	19
31.1.22	14	22	64	14
7.2.22	28	14	95	22
14.2.22	31	16	88	17
21.2.22	57	21	87	15

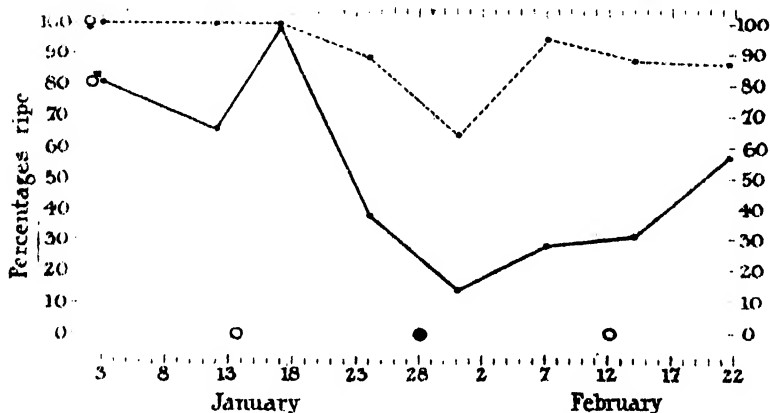


FIG. 7.—State of maturity of *Mytilus* sp. at Alexandria, January-February, 1922. Curve ♂ gives the percentage of males in which the teased testis showed spermatozoa only, without admixture of spermatocytes; curve ♀ gives the percentage of females in which the ovary contained numerous eggs. The dates of full and new moon are marked by white and black circles respectively.

Oysters from Alexandria (*Ostræa* sp.) were accordingly examined, but in this instance I had to be content with a macroscopic comparison of bulk, for the work was done in October-December, a period when the oyster at Alexandria is not breeding. On each of six occasions, at 10 days' interval apart, three dozen oysters were removed from their shells and preserved in formalin. The different lots were afterwards compared, but no regular variation in bulk was detectable. Although this method is admittedly crude, yet it must



be remembered that the supposed lunar variation is sufficient to catch the eye of fishermen and cooks. Prof. T. C. Nelson, whose work on the American oyster (*Ostrea virginica*) was quoted above (31), writes to me, speaking of this form: "We have no evidence of lunar periodicity. As soon as the temperature reaches 68° to 70° F. and remains there for a few days the oysters begin spawning."

(b) *Crabs*.—Crabs form the last of the list of animals about which a belief in a lunar variation in bulk exists in Egypt. The species eaten (*Neptunus pelagicus*) is said to be full of flesh (muscle) at full moon and empty at new moon. It was inherently improbable that any truth would be found underlying this statement, since variations in the state of the internal organs of crustacea are largely dominated by the moults, which occur frequently in early life and at less frequent intervals as the animal ages. There could not then be any regular monthly variation throughout life. However, since the statements about *Centrechinus* had turned out so unexpectedly to be based on truth, crabs were examined from the same point of view.

The specific gravity of the right great chela of a number of *Neptunus pelagicus* was determined on five different dates. The data are given in Table IX. On each occasion the crabs were purchased at 7 a.m. in the Suez fish-market

Table IX.—Showing the Average Specific Gravity of the Right Chela of Male *Neptunus pelagicus* at Suez on Certain Dates.

Date.	Days after new moon.	Numbers examined.	Average specific gravity.
6.9.21 .....	4	20	1.22
9.9.21 .....	7	47	1.22
17.9.21 .....	15	44	1.22
25.9.21 .....	23	36	1.24
30.9.21 .....	28	51	1.23

and the examination was made at 10 a.m. Males alone were examined. Specimens which had just moulted and consequently had soft exoskeletons were rejected. The chela was always cut off at the same point of its base. The Table shows that in fact there is no correlation between the specific gravity and the phases of the moon.

It is nevertheless remarkable that very considerable variation exists between the specific gravity of different individuals, amounting sometimes to 10 per cent. of the mean specific gravity. The differences are found equally in chelæ with thick and with thin exoskeletons—they depend upon variations in the development of the muscles, and are evident in extreme cases to the naked

eye upon cutting open the chela, which can be seen to contain well-developed or poorly-developed muscles. There is no correlation between the specific gravity and the size of the crab.

6. *Lunar Reproductive Periodicities already described in Animals.*

(a) *Polychaetes*.—Up to the present the best known case of lunar reproductive periodicity has been that of a polychaete, the Palolo worm (*Leodice viridis*\*), living in Pacific coral reefs. At the last quarter of the moon in October and November the posterior parts of the worms, laden with genital products, become detached from the anterior portions. While the latter remain among the coral, the genital segments swim up to the surface where they shed their spermatozoa and eggs. This swarming takes place at low tide in the early morning on several successive days, the swarms being composed of enormous numbers of individuals which die after having spawned. The stimulus for swarming, whether moonlight, tide or some other physical change depending on the moon, is unknown. Good accounts are given by Friedländer (7), Woodworth (43) who adds a full bibliography, and Corney (5).

In the Atlantic another Palolo (*Leodice fucata*) swarms usually at the 3rd quarter of the June-July moon, but if this falls late in July there is, in addition, an earlier swarm at the first quarter (Mayer, 27, Treadwell, 40, p. 45). Mayer (28, 29) was the first to seek a cause for lunar reproductive periodicity experimentally. He placed 11 mature worms in a floating (i.e., tideless) box 30 days before the swarming time was due. Only 4 of these worms swarmed, whereas in nature all mature individuals are said to swarm. Thus, the tide appears not to be the sole cause of swarming, unless those worms which swarmed in the floating box did so owing to a tidal rhythm previously acquired, as in the case of the reproduction of *Convoluta* mentioned above (p. 536). Mayer also put 22 worms in floating boxes protected from moonlight. None of these animals swarmed. The light seems therefore to be a necessary contributory cause of swarming. But Treadwell obtained a contrary result (41). These experiments should be repeated with more individuals.

Lunar periodicity in the swarming of polychaetes also occurs in the Nereidæ. *Ceratocephale osawari* has been described by Izuka (13, 14, 15) as swarming in Japan four times a year, in October and November, at both full and new moons. Here the bi-lunar periodicity is presumably an effect of the spring tides, but we are ignorant of how the greater tidal range may react on the animals.

\* The Palolo worms have received several changes of name. I follow Treadwell (40).

At Woods Hole *Nereis limbata* swarms after sunset from June to September (Lillie and Just, 24). The numbers appearing nightly begin near the time of full moon, increase to a maximum, sink to a low point about the 3rd quarter, increase again, and then decrease to vanish after new moon. At the same place *Platynereis megalops* swarms nightly during the waning moon while none appear at the surface between new and full moon (Just, 16). At Naples the swarms of *Nereis dumerilii* are described by Hempelmann (11) as centering about the 1st and 3rd quarters. None are caught at new or full moon. The latter bilunar, i.e., apparently tidal, periodicity is remarkable, since the tidal range at Naples is much less than at Woods Hole, yet swarming of *Nereis* at the last-mentioned place is not correlated with the tides.

(b) *Fish*.—A case of spawning in fish correlated with the moon's phases has lately been described from California by W. F. and J. B. Thompson (39). On the 2nd, 3rd and 4th nights after the full moons of March, April, May and June, a smelt (*Leuresthes tenuis*) comes in-shore in swarms. The sexes pair and burrow in the sand at the wave-margin, where the eggs are buried. The causes of this behaviour are unknown. The authors note (39, p. 74) that "the migratory impulse does not arise in response to the light of the moon, for during the last spawning run in May heavy clouds obscured the moon through each night."

(c) *Man*.—Arrhenius (2) has shown statistically that there exists a low correlation between the frequency of human births and the sidereal lunar period of 27.32 days.\* The same kind of periodicity was found for menstruation, but to a more pronounced degree.

### 7. Lunar Periodicity in Plants.

Among plants the sole authentic case of a lunar rhythm in reproduction seems to be among the algæ. The marine *Dictyota dichotoma* at Beaufort, North Carolina, produces one crop of sexual cells in each lunar month (Hoyt, 12). The same species at Bangor, Plymouth (Williams, 42) and Naples (Lewis, 23) has a tidal reproductive rhythm, i.e., two cycles per lunation. This difference in behaviour is unexplained and is the more problematical since the average tidal range at Beaufort is small (0.8 metres) resembling that at Naples (0.3 metres) and differing widely from Bangor (5.4 metres). *Sargassum* (Tahara, 37) and *Nemoderma* (Kuckuck, 22) also show bi-lunar reproductive cycles.

Popular beliefs in a favourable influence of the moon on plant growth are

\* 27.32 days is the time taken by the moon to make one circuit of the heavens. The synodical period, or lunation, of 29.53 days is the time in which the moon goes through her phases.

world-wide and ancient. In particular it is stated both by classical authors and by the Egyptian fellahin of to-day that melons, marrows and other fruits of the Cucurbitaceæ grow most rapidly on moonlight nights. To test this belief, I made daily measurements of the fruit-length of a small variety of marrow (*Cucurbita pepo*) in Cairo. The results of the observations, which extended over two lunar months, are shown in fig. 8. Each curve is smooth

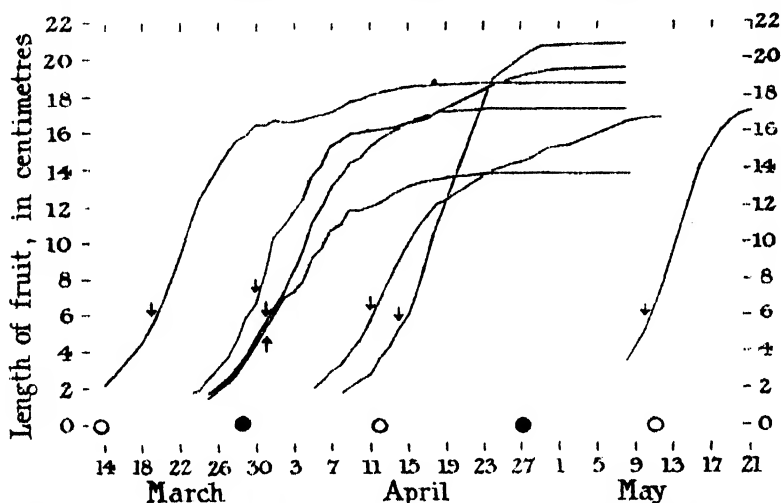


FIG. 8.—Growth-curves of the fruits of *Cucurbita pepo*. Each curve refers to one fruit. The arrows mark the dates of flowering. The flowers last a few hours only. Before the appearance of the flowers the measurements refer to the ovary-length. Full and new moons are indicated by white and black circles respectively.

and S-shaped, of the form characteristic of autocatalytic reactions, and there is no differential rate of growth at different parts of the lunar month: the slopes of the curves do not vary according to the moon's phases. As the lunar influence is supposed to show itself especially in these cucurbitaceous fruits, it may safely be assumed to be absent in all fruits.

There are, however, two demonstrated effects of the light of the moon upon plants. Firstly, Musset (30) showed that some flowering plants are positively phototropic to moonlight. Secondly, Loftfield (26) states that at night stomata open in moonlight. The consequent periodic opportunity for increased transpiration and respiration might cause other rhythmic changes in the plant, with a lunar period.\*

\* Since the commencement of the present MS. a letter has appeared in "Nature" (vol. CXL, p. 49) from Miss Semmens, announcing (1) that moonlight increases the velocity of germination of seeds, and (2) that polarised light increases the rate of hydrolysis of starch with diastase. It is suggested that (2) may be an explanation of (1). But see p. 536 above.

Moonlight may cause photosynthesis. The amount would be so small that no intake of  $\text{CO}_2$  from the surroundings by the plant would occur, but the  $\text{CO}_2$  output due to respiration would be less in moonlight than in darkness. Whether the extent of this diminution in  $\text{CO}_2$  output would be detectable or not would depend upon the delicacy of the means of detection used. Now, it becomes of importance to determine if this actually occurs, for the following reason. Kofoed (20) found a lunar periodicity in the frequencies of plankton organisms in the Illinois River. W. E. Allen (1) found indications of the same thing in the San Joaquin River, California. The maximum frequency of algae occurred at full moon, that of crustacea a little later. While the crustacean presumably follows the algal maximum because the animals feed on the plants, Kofoed attributes the algal maximum occurring about full moon to a photosynthetic effect of moonlight. He supports this hypothesis by reference to experiments of Knaute (21), who states that he found the oxygen-content of water containing *Euglena* to be higher in moonlight than in darkness, which means that moonlight causes photosynthesis. Knaute's figures show a photosynthetic effect of moonlight with a ratio to that of sunlight of 2 : 9. This is a surprisingly high ratio, since the intensity of the light of the sun is about 600,000 times that of the full moon.

I have in part repeated Knaute's work at Cairo, measuring the change in H-ion concentration in the water in which a plant was placed, due to the change in its  $\text{CO}_2$  content consequent upon photosynthesis or respiration.

A measured length of *Elodea* was placed in a corked test-tube filled with water containing brom-thymol blue, which is not adsorbed by the plant and is non-toxic. The  $P_H$  changes were noted at stated intervals (the water having first been charged with  $\text{CO}_2$  for photosynthesis). It was found that four hours' exposure at  $27^\circ \text{C}$ . to full moonlight caused no alteration in the  $P_H$  decrease due to respiration in darkness. The optimum light intensity for photosynthesis was then determined and it was found that an hour's exposure of the *Elodea* to this light gave a  $P_H$  increase of 0.45 : 4 hours would therefore (assuming the rate of photosynthesis to remain uniform) give a  $P_H$  increase of 1.80. The same length of time in darkness caused a  $P_H$  decrease of 0.12, so that tubes initially at the same  $P_H$  and kept for four hours respectively in the optimum illumination and in darkness would differ at the end by  $1.92 P_H$ . The minimal detectable  $P_H$  difference by the method used was 0.025, so that if there was any photosynthesis due to the 4-hour exposure to moonlight it caused a  $P_H$  change  $< 0.025$ . Thus, any photosynthetic effect of moonlight is less than  $0.025/1.92 = 0.013$  that of sunlight. This is very different from Knaute's

figure and unless algæ behave differently from *Elodea* the amount of lunar photosynthesis, if any, is small.

In conclusion, I wish to thank Prof. Edward Hindle of the School of Medicine, Cairo, for his valuable advice and assistance ; M. Henri Roger, for continual help ; Drs. Bonan and Cano of the Quarantine Laboratory, Port Taufiq, for their hospitality ; M. Dormoy, Chef de Section of the Suez Canal Company, for generous collecting facilities ; Mr. G. W. Paget, Director of Egyptian Fisheries Research, for help in obtaining material ; Mr. Clifford Dobell, F.R.S., for finding a number of valuable classical references for me ; and numerous correspondents.

#### 8. Summary of Conclusions.

1. A belief in a variation in the bulk of marine invertebrates which serve as articles of food, correlated with the lunar period, was general in ancient Greece and Rome and persists to-day in countries bordering the Mediterranean and at Suez. It is shown in this paper that, while the belief is based on fact as concerns sea-urchins in the Red Sea, it is false regarding sea-urchins in the Mediterranean and other invertebrates in both seas. It is suggested that in ancient Egypt the belief spread from Suez, passed to Greece, and has persisted in Mediterranean countries until to-day.

2. The details of the lunar reproductive cycle of the echinoid *Centrechinus* (*Diadema*) *setosus* at Suez are described. At each full moon during the breeding season spawning takes place. After this a fresh crop of genital products is developed, to be spawned at the ensuing full moon. One and the same individual may become sexually mature at consecutive lunar periods.

3. There is no lunar reproductive periodicity in the echinoid *Strongylocentrotus lividus* at Alexandria, Naples or Marseilles ; nor in the mussels *Mytilus* *sp.* at Alexandria and *Mytilus variabilis* at Suez. These forms, however, show evidence of simultaneous though non-periodic spawning.

4. There is no lunar periodicity in the rate of growth of fruits.

5. The possibility of lunar photosynthesis is tested.

[Note added November 5, 1923.—The following points bearing on the lunar reproductive periodicity of echinoids have been worked out at the Roscoff Biological Station, Brittany, between June and August of this year.

(1) The state of maturity of *Strongylocentrotus lividus* was examined. Fig. 9 shows that there is no lunar periodicity : the behaviour, then, is identical with that of the same species at Alexandria, Naples and Marseilles. This

supports the view (p. 536) that tides are not the cause of the *Centrechinus* periodicity at Suez, for while tides are extremely small at Alexandria they are big at Roscoff. Fig. 9 shows evidence of the same simultaneous but non-periodic spawning as does fig. 4.

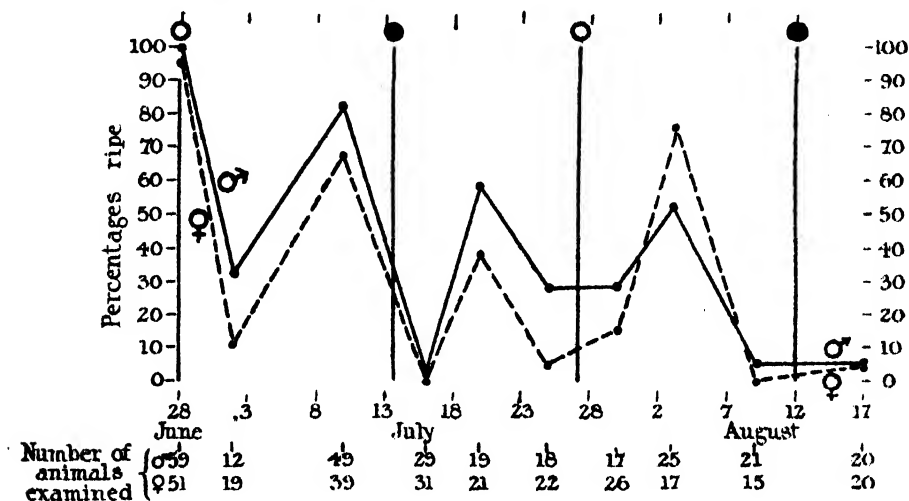


FIG. 9.—State of maturity of *Strongylocentrotus lividus* at Roscoff in 1923. Curve ♂ shows the percentages of males in which the teased testes gave spermatozoa only, without admixture of spermatocytes; curve ♀ shows the percentages of females in which the ovaries contained ripe eggs only. The dates of full and new moon are indicated by white and black circles respectively.

The *Strongylocentrotus* of fig. 9 were fished in a depth of 2·5–5·5 metres, each time at the same place. On August 9 individuals taken 650 metres distant from this place, in 12 metres of water, were mostly ripe. Thus the state of maturity of individuals in contiguous areas is different.

(2) I have found that the oxygen-consumption of pigmented animal tissues is greater in light than in darkness. If moonlight has sufficient intensity to cause the effect, this may be the reason for the lunar reproductive periodicity of *Centrechinus*. This investigation is in progress.

(3) With regard to the mechanism and causation of spawning in echinoids, our ignorance of which was referred to on p. 536, the following points have been noted: (a) The extrusion of genital products is due to the contraction of muscles in the gonad walls, more abundant in the ovaries than in the testes. These muscles can be artificially stimulated to cause spawning by the touch of a camel's hair brush. (b) The spawning of a male causes the spawning of all other ripe males and ripe females in the neighbourhood.

(4) The rate of development of genital products in *S. lividus* has been investigated (cf. p. 530). At intervals of a few days windows were made in the tests of individuals kept in an aquarium. A piece of gonad was removed for examination through each window, which was then closed with wax (Koehler, 19, p. 127). "Spent" females containing no eggs developed ovaries full of ripe eggs in nine days. The water temperature was 18°.

Miss Viola Eustice, working at Southampton under my direction from August to October of this year, has shown that *Mytilus edulis* has no lunar reproductive periodicity. This accords with the behaviour of *Mytilus* at Alexandria and Suez (p. 538).]

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*Studies on the Sex-Ratio and Related Phenomena.—I. Fœtal Retrogression in Mice.*

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*Introductory.*

During recent years an increasing amount of evidence has been accumulated relative to the death and retrogression of embryos and fœtuses. Pre-natal mortality resulting in abortion is a phenomenon of obvious occurrence, but the internal removal of dead fœtuses and embryos is quite another matter. Most of the records which exist on this latter process relate to polytocous animals, in which the question is obviously different from monotocous animals. In multiple pregnancies the abortion of a dead fœtus could only take place at the sacrifice of the rest of the fœtuses, and such being the case a means of removing the dead fœtus without terminating the pregnancy would be a considerable advantage to the animal. In monotocous animals cases of fœtal retrogression appear to be less common, though this may be accounted for by the increased difficulties of observation.

The supposition of re-absorption has been challenged on the grounds that decomposition could hardly proceed in company with gestation. It seems, however, that in some animals certain products of gestation are normally assimilated by the maternal organism. Robinson (15) says that the maternal tissues are not shed at the time of birth in the mole, and that some of the fœtal tissues are retained to be absorbed later. Jenkinson (4) also states that in *Peromyscus* the allantoic blood vessels are regularly absorbed through the agency of maternal leucocytes by the parturient uterus and that fœtal tissues are absorbed somewhat similarly in *Dasyurus*. Also the order of the disappearance of the conceptus is suggestive. Meyer (14) says that the embryo

or foetus is the first member of conceptuses to be removed, and my own observations show that the membranes are intact long after decomposition has started in the foetus. This strongly suggests that the process is one of organized absorption rather than of putrefactive activity, which would presumably begin with the membranes.

Considering also the frequent occurrence of degenerate foetuses along with normal ones, it is impossible to deny that foetal death can be followed by retrogression and partial absorption as well as by abortion. It is not possible to draw a definite line between absorption and abortion. Young embryos can apparently be entirely absorbed, whereas retrogression of older foetuses results in the abortion of macerated remains. Such *débris* may, however, be retained till the birth of the normal foetuses and pass out with the non-foetal products of gestation. In this connection Hammond (3) says, "Foetal atrophy is quite general. The reason why it has not been observed more frequently is that absorption takes place either entirely, or that the atrophic foetus is so-reduced at birth that it passes unnoticed in the cleansings."

The following quotation is from King (6): "Faulty implantation is responsible for the abnormal development of many ova in the rat; these ova as a rule die at early stages and are absorbed *in situ*." Many more cases are on record of foetal retrogression and absorption, but more details cannot be given, except to say that such processes have been found to occur in the mare, cow, sheep, goat, guinea pig, hamster, rat, mouse, ferret, dog, cat, mole, rabbit, and pig.

Considering this wide range of mammals, the question of the actual amount of retrogression is of great interest, but only two cases are known to the writer where the amount of death not involving abortion has been computed. Hammond (3) found in 80 pregnant ewe sheep 116 corpora lutea and 101 normal foetuses, and 8 atrophic ones in various stages of re-absorption, 7 ova being unaccounted for. In a number of pigs the same author found 396 corpora lutea, 267 normal foetuses and 49 atrophic ones. It is justifiable to assume that very few ova would escape fertilisation, especially in the case of the pig, and the total mortality, therefore, works out at 12.9 per cent. in the case of the sheep and 32.5 in the case of pigs. In the case of the rat, Long and Evans (11) found that the average size of litter was 6.7. In 50 animals dissected just after ovulation and copulation the average number of eggs in the Fallopian tubes was 9.6, and the average number of corpora lutea was 10. These observations argue a very high rate of mortality in the rat.

*Objects.*

It appears, therefore, that while a considerable amount is known of the occurrence of fœtal retrogression, little has been found of the actual amount, and further data, relating to mice, are here presented. Secondly, nothing appears to have been found regarding the sex incidence of fœtal death which results in re-absorption. Owing to the fact that many authors have shown that abortion falls preponderatingly upon the males, it appeared that any information obtainable relating to the sex incidence of the other type of fœtal elimination would be of interest.

*The Amount of Retrogression in Mice.*

For the work here described it was assumed that if the doe had been in continuous contact with the male the eggs would probably all be fertilised. Pregnant does were killed about a day before parturition was due and the ovaries serially sectioned for the corpora lutea. In this way it was found that 82 ova had gone to the production of 74 full-time fœtuses, there being 8 unaccounted for. This means that the loss of embryos per 100 normal full-time fœtuses was 10·8. As these animals were bred under normal conditions this may be taken as the normal amount of mortality. It may be mentioned that in some cases the most remarkable variation in the size of the surviving conceptuses was found. Further experiments were conducted as follows: Daniel (1) and also King (5) have shown that in mice and rats the doe may become pregnant at the œstrus period which follows within 24 hours after parturition, and that the gestation period of the second litter is prolonged in some cases as much as ten days. From his experiments, Daniel concludes that "the period of gestation in lactating mothers varies directly with the number of young suckled." King (5) has reported a similar fact about rats. Kirkham (7) confirmed the facts on mice and showed that the delay was due to the failure of the embryos to embed themselves in the uterine mucosa. The cause of this non-implantation he considers (8) to be inhibition due to the coincident functioning of the mammary glands of the mother. Kirkham also shows that though the prolongation of the second gestation cannot be exactly correlated with either the number of young removed or the time of removal, leaving the young for more than six days regularly delayed implantation.

The blastocysts presumably always have to contend with unfavourable conditions in a uterus which has just concluded a pregnancy. This would suggest an added embryonic mortality; and it also occurred to the writer that

the abnormal prolongation of the blastocyst stage, due to inhibited implantation, could be used experimentally to accentuate the hardships arising from the proximity of the previous pregnancy, and thus to determine the effect of unusual hardship upon embryonic and foetal mortality.

In the experiments I made on these lines the males were allowed to stop with the females for 24 hours after the birth of the first litter, and if the doe "held" she was dissected when the second parturition became imminent. The second insemination was successful in about 50 per cent. of the cases. After receiving the male subsequent to parturition, the does were divided into two classes. In the first the previous litter was allowed to remain for intervals up to six days, in the other class the suckling was allowed to go on into the later stages of the second pregnancy. As before, the number of corpora lutea was counted and used as a criterion of the number of eggs discharged.

A complication arises, however, owing to the fact that the corpora lutea from the first litter became confused with those of the second litter, which are usually known as the corpora lutea of lactation (Long and Evans, (12)), when a second pregnancy does not occur. In these circumstances, therefore, there appeared only two courses, vital staining of the first set of corpora or comparing the total number of corpora with the total number of foetuses represented, namely, the total of both first and second litters. The latter alternative was decided upon as being the most simple, and only having the disadvantage of representing the result in a diluted form. There is no reason to suppose that the first litters have a different uterine mortality ratio than was found normally, and thus any variation from normality in the difference between the total of the first and second litters and the number of corpora lutea would be caused by anomalies in the embryonic and foetal elimination during the second gestation. In any case, as the number of young in the previous litter is known in each case, it is possible to calculate the normal number of corpora lutea belonging to it, and the consequent number belonging to the second litter.

Table I.—Number of Corpora Lutea and Foetuses from Suckling Mothers.

	No. in previous litters.	No. of second litters.	No. in second litters.	Corpora lutea.	Difference between total foetuses and total corpora lutea.
Class I .....	29	4	22	58	7
Class II .....	17	4	31	57	9

In the first class the fœtuses in the two litters total 51, as compared with the 58 corpora lutea found. The first litters accounted for 29 of these fœtuses, and on the basis of the experiment on the normal amount of elimination we should expect that the loss of ova on these 29 fœtuses would be  $29/1 \times 10.8/100 = 3.13$ . We find, therefore, that 32.13 corpora lutea would be required to produce the 29 young of the first litters. This means that 25.87 corpora lutea are left to account for the 22 fœtuses of the second litters. Thus the loss on these 22 fœtuses is 3.87 ova, which gives a loss per 100 fœtuses of 17.6, which is 75 per cent. higher than the normal.

In the second class the normal loss on the 17 fœtuses of the previous litters will be  $17 \times 10.8/100 = 1.84$ . Since the loss on the whole 57 fœtuses is 9 ova, the loss on the 31 fœtuses of the second litters must be 7.16. This gives  $7.16 \times 100/31 = 23.1$  ova loss per 100 normal fœtuses, which is more than double the normal.

These experiments may be summed up as follows. There is a normal elimination of fertilised ova, due to other causes than abortion, as this would terminate the pregnancy entirely. This normal loss amounts to 10.8 ova per 100 normal fœtuses found. If, however, conception takes place when the uterus is disturbed by a recent parturition, the loss is increased to 17.6 per 100 fœtuses; and when these unfavourable conditions are accentuated by prolonged suckling of the first litter the elimination ratio rises to 23.1.

It is to be gathered, therefore, that bad conditions during implantation can readily increase the amount of embryonic elimination, which is normally quite appreciable.

#### *The Sex Incidence of Retrogression in Mice.*

The problem of assessing the sex-ratio of embryos and fœtuses which disappear by absorption is very difficult, and so far as the writer knows has not been attempted previously. An indirect endeavour may, however, be made. It has been shown above that three degrees of the amount of fœtal elimination can be arrived at, and at the same time as the above experiments were carried out the sex-ratio at birth of litters produced under corresponding conditions was ascertained. In over 1000 mice bred under normal conditions the sex-ratio was found to be 118 males per 100 females, whereas in litters produced by suckling mothers the results were as follows:—

Class I (previous litter suckled less than six days) produced 14 litters with 92 young, and of these 41 were males and 51 females, giving a sex-ratio of 80.4. In Class II (previous litter suckled six days or more) 7 litters with

47 young were produced, and of these 18 were males and 29 females, giving a sex-ratio of 62·1. These results may be summarized in a table :—

Table II.—Sex-ratio and Amount of Embryonic Elimination in Mice

	Loss of ova per 100 fetuses.	Sex-ratio. Males per 100 females.
Normal .....	10·8	118·0
Previous litter left less than six days .....	17·6	80·4
Previous litter left more than six days .....	23·1	62·1

A diagram brings out very well the inverse proportion between the sex-ratio and the amount of elimination.

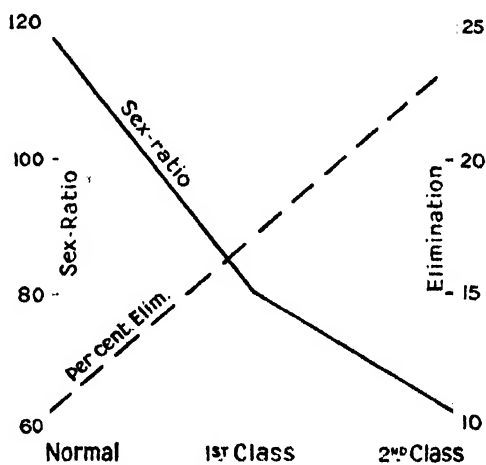


FIG. 1.

From these results it is almost impossible to doubt that prenatal mortality and retrogression falls most heavily upon the male fetuses.

### Discussion.

While it is not unexpected that the amount of foetal mortality increases under bad conditions, it is hard to see why the males should be most affected. Any explanation of this fact can only be attempted by considering the various possible causes of pre-natal mortality and the consequent possibility of selective action between the sexes. The causes of foeta retrogression are not known with any certainty. We owe to Hammond (2, 3) the

suggestion that adiposity, in-breeding, or a lethal factor similar to that demonstrated by Kirkham (9) in the homozygous yellow mice, may account for embryonic mortality. Marshall (13) points out that it is probably not due to over-crowding. Some of the mortality is no doubt due to malnutrition. Why the male mortality should be greater than the female is difficult to understand, but if there were any inherent difference in vitality between embryos of the two sexes a selective action might be set up. In this connection Marshall says: "Nutrition is probably an important factor both in the size of the litter and in the size of the individuals, as experiments on various animals have shown, but there must be variation in the degrees of vitality inherent in different foetuses." Lillie (10) suggests that the greater mortality in the male foetuses is the result of disturbance of the equilibrium which protects the males from the sex hormones of the mother. None of these suggestions seems to be quite satisfactory, and the probability is that the observed result is brought about by a variety of causes.

#### *Summary.*

1. A considerable amount of evidence exists to show that foetal death may be followed by retrogression *in utero* as well as by abortion.
2. In only two cases, however, had the amount of such mortality been estimated. Hammond, for pigs and sheep, and Long and Evans, for rats, have shown the amount to be very considerable.
3. In the experiments here described it was found that under normal conditions the amount of foetal retrogression without abortion was 10·8 per 100 full-time foetuses. In pregnancies following immediately on previous ones the amount was found to be raised to as much as 23·1.
4. In correlation with this increase the sex-ratio at birth was found to drop from the normal 118·0 males per 100 females to 62·1.
5. The unavoidable inference is that pre-natal mortality falls preponderatingly on the males, though it is difficult to see why this should be so.

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### *The Relation between the Phosphate in Blood and Urine.*

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(From the Biochemical Laboratory, Cambridge.)

No experiments appear to have been made on the changes which occur in the blood of man after the ingestion of phosphate. In experiments on the dog, Denis (1) has shown that after administration of phosphate amounting to 260 mgms. P/kilo weight, there is no detectable change in the phosphate of the blood, although the increased excretion of phosphorus in the urine indicates that much of the salt has been absorbed. This was attributed to rapid excretion by the kidney. Very little is known of the excretion of phosphorus by the human kidney. The relation between the concentration in the blood and the urinary excretion when associated with either an acidosis or an alkalosis is described by Haldane, Wigglesworth and Woodrow (16). The present investigation was carried out mainly to determine the response

of the kidney to a simple increase in the concentration of phosphate in the blood uncomplicated by reaction changes.

We have further investigated the question of the distribution of inorganic phosphate between the plasma and the corpuscles of the blood ; under normal conditions and also after phosphate ingestion. Some experiments have also been made to determine whether it is possible to increase the normal organic store of phosphorus in the blood by administration of the inorganic salt.

#### METHOD.

The method used for the determination of the inorganic phosphorus in blood and urine in the following experiments was the modification of the Bell-Doisy (2) colorimetric method recently devised by Briggs (3). For the present purpose, its chief merit lies in the fact that as only 2 c.c. of blood are required for each estimation, serial determinations on blood from a finger prick are rendered possible. Briggs' method has been tested by Eddy and Heft (4) among others and found to give identical results with Tisdall's method (5). As duplicate determinations made on several occasions never differed by more than 4 per cent. and usually by considerably less, only one estimation was generally made on each sample. A Kober colorimeter was used for the estimations. A minimal amount of finely ground potassium oxalate was used as an anticoagulant. Briggs has stated, and we have confirmed, that the small quantity of oxalate used does not interfere with the estimation. The removal of the proteins is carried out exactly as described by Briggs, with the exception that the greater part of the precipitate is removed by centrifugalisation before filtering. By this means the time taken by the procedure is very much shortened ; and it has been possible so to standardise the technique that each estimation is carried out in 1 hour 5 minutes  $\pm$  2 minutes from the time of drawing the blood. This last point is important since the majority of our estimations have been made upon whole blood ; and it has been shown by Zucker and Gutman (6) that the value obtained for the inorganic phosphorus content of the blood by the method of Bell and Doisy varies appreciably with the time elapsing between the drawing of the blood and the final estimation. For example, they state that the inorganic phosphate in the whole blood is identical with that in the plasma if the estimation is completed within half an hour from the time of drawing the blood ; but that if 1 hour is allowed to elapse, the whole blood yields a value 0.5 mgms. per cent. higher than that given by the plasma. This is due to the hydrolysis of a portion of the organic (acid soluble) phosphorus

of the blood with consequent liberation of more inorganic phosphorus. The colour development in Briggs' method is much slower than in the original Bell-Doisy method, and this may be the explanation of the fact that we obtain identical values for the phosphate in plasma and the whole blood although the procedure occupies rather more than an hour.

Since this work was done Buell (7) has claimed that in dogs there is no inorganic phosphorus in the corpuscles, the whole of the inorganic phosphate of the blood being confined to the plasma; and that in human blood, although the corpuscles have never been found free from phosphate, yet only very small amounts are present. These small traces, in her opinion, are attributable to imperfections in the technique used. She accounts for the identical values in plasma and whole blood obtained by Zucker and Gutman (6) as due to the hydrolysis of some of the organic phosphorus in the corpuscles. When we made simultaneous determinations on the whole blood and plasma (Table I), we found the same values, although the absolute amount varied greatly. The only exception to this condition, as will be discussed later, is when the value in the plasma rises far above normal.

It is difficult to reconcile these figures with the view put forward by Buell. Since the time taken for each of these estimations was the same, it would be expected that the same degree of hydrolysis of the organic phosphorus compounds would take place. If, for the sake of simplicity, we assume equal volumes of plasma and corpuscles in the blood, then within the same length of time, the amount of hydrolysis must have varied from 4.0 to 0.83 mgms. phosphorus per 100 c.c. of blood.

Table I.—Values in milligrams P per cent.

Whole Blood .....	3.34	3.46	3.43	1.91	3.87	3.03	3.31	3.54	4.00	3.28	2.78	0.83
Plasma .....	3.40		3.43	1.86	3.77	2.91	3.31	3.66	4.08	3.16	2.73	0.96

The values obtained by us for the inorganic phosphorus of normal human blood are in agreement with those of others, the values varying in different individuals and in the same individual at different times from 2.5 to 3.5 mgms. P per 100 c.c.

The method of incineration described by Bell and Doisy (2), has been used for the estimation of the total acid soluble phosphorus in blood.

This method has recently been criticised by Baumann (8), who proposed hydrogen peroxide as an alternative oxidising agent. We have tried both methods and have found Bell and Doisy's original method more satisfactory. When tested by incinerating known amounts of a standard solution of potassium phosphate the loss has never exceeded 5 per cent., and duplicate determinations have always shown excellent agreement.

#### EXPERIMENTAL.

##### 1. *The Excretion of Phosphate by the Kidney.*

We have taken doses of the acid and alkaline sodium phosphates containing amounts of phosphorus varying from  $1\frac{1}{2}$  to 2 grms. Similar changes in blood and urine were obtained with either salt. Early experiments seemed to indicate the possibility of a maximum concentration of phosphate in the urine having been attained. This question was investigated by taking larger doses of the salt and by abstaining from water during the course of the experiment. Typical results are given in figs. 1 and 2.

A light breakfast as free from phosphate as possible was taken about 8 a.m. Thereafter the subject took no food or water until dinner at 7.45 p.m. 25 grms. of alkaline sodium phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) were taken about 10.15 a.m. and blood and urine samples obtained at frequent intervals during the day. It will be seen that the inorganic phosphate of the blood increases by 50 to 60 per cent. of its normal level, usually attaining the maximum value within 2 hours of the ingestion of the salt. The concentration then falls very gradually and remains above normal throughout the day. It is well known that phosphates are absorbed with difficulty from the intestine, and probably the gradual fall obtained is attributable to this fact. The curve of the hourly excretion of phosphorus in the urine is seen to run more or less parallel to that of the blood phosphate; but whereas the concentration of the blood phosphate only increases by about 50 per cent., the urinary output rises to about five times its normal value. (In fig. 2 it will be observed that the fall in output which occurs normally in the morning makes its appearance before enough phosphate has been absorbed to mask the effect. This diurnal change has been discussed most recently by Fiske (9).) Meanwhile, the concentration of phosphorus in the urine rises gradually from three times to eighty times that in the blood. The highest concentration we have ever reached was rather more than 0.1 molecular, or 80 times that of the plasma. There was no indication that this was the maximum concentration of which the kidney

is capable. Much higher concentrations were found by Ambard (10) for urea and by Davies, Haldane and Peskett (11) for chloride and bicarbonate.

In many of the experiments we have done in conjunction with Haldane on experimental acidosis and alkalosis (16), a marked diuresis has been associated with changes in the concentration of phosphate in the blood. Accordingly, we have carried out experiments to determine how far the excretion of large

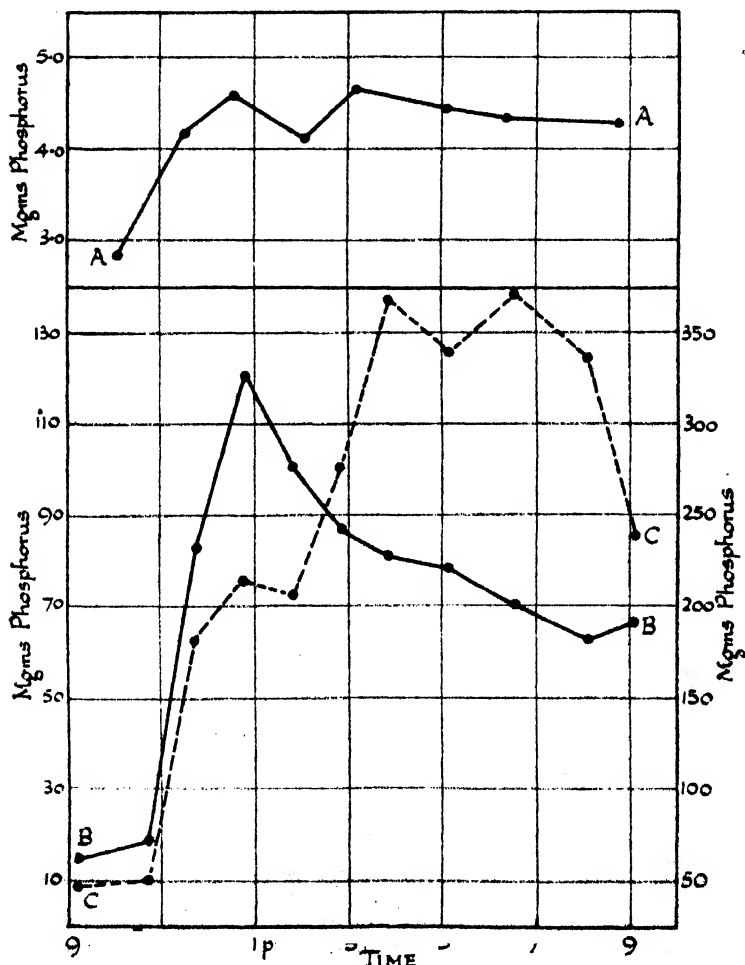


FIG. 1.—Subject V.B.W. 25 grms.  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  taken in 175 c.c. of water at 10.20 a.m. Dinner 7.45 p.m. Curve A.—Mgms. Inorganic Phosphorus per 100 c.c. of Blood. Curve B.—Rate of Excretion of Phosphorus in the Urine in Mgms. per Hour. (Left-hand scale.) Curve C.—Concentration of Phosphorus in the Urine in Mgms. per 100 c.c. (Right-hand scale.)

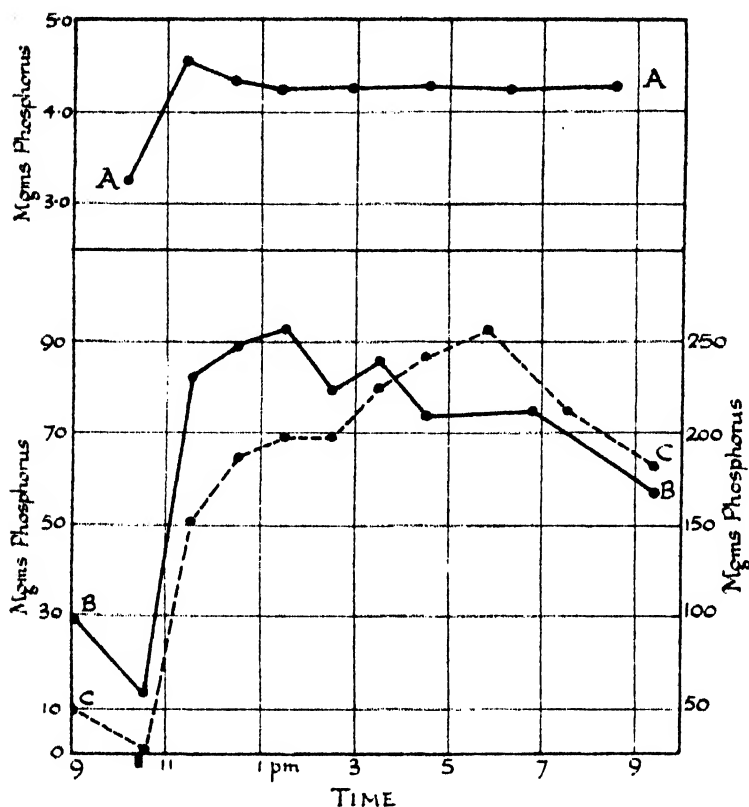


FIG. 2.—Subject C.E.W. 25 grms.  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  taken in 170 c.c. of water at 10.20 a.m. Dinner 7.45 p.m. Curves A, B and C as in fig. 1.

quantities of water affects the phosphate output. Typical results are summarized in figs. 3 and 4.

The experiments were carried out exactly as described above, except that a diuresis was produced by drinking about  $2\frac{1}{2}$  litres of distilled water during the day. The concentration in the blood reaches a slightly higher value than on the days when no water was taken. In all probability this is due to the better absorption of the salt from the intestine under these conditions. The gradual fall occurs as in the previous experiments. The curves of urinary output also closely resemble those of the previous experiments and are unaffected by the very great changes in the volume of urine secreted. In fig. 3 there is an apparent fall in the excretion after the subsidence of the first diuresis. This, however, was really due to the inability to empty the bladder completely after the very great distension of the preceding hour.

The total excretion of phosphorus in the urine in one experiment on a 'dry' day when 2.1 grms. of phosphorus were taken was 1.7 grms., and on a

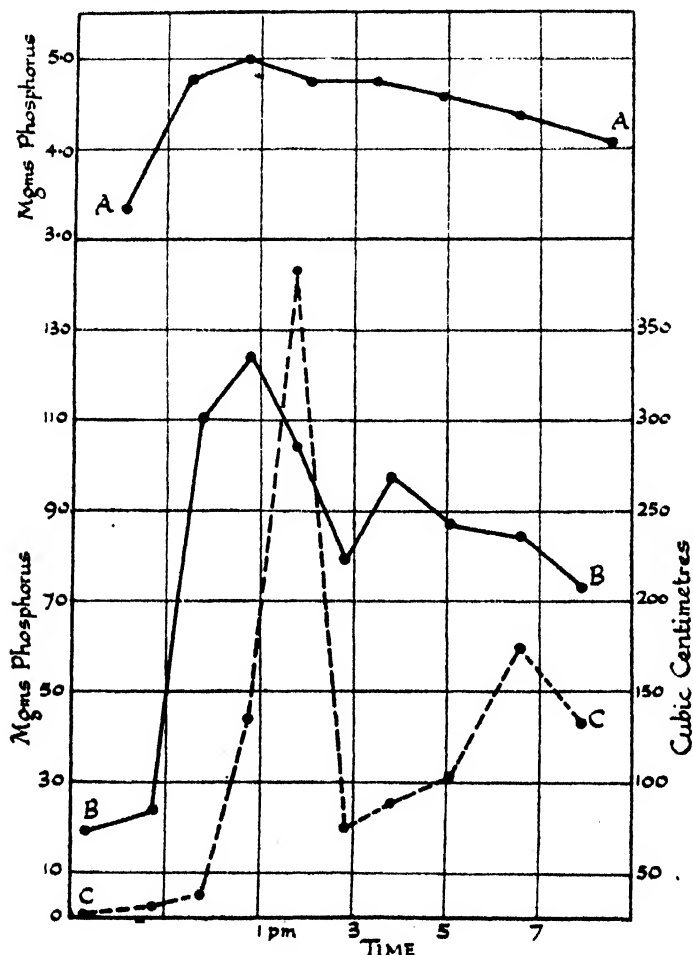


FIG. 3.—Subject V.B.W. 25 grms.  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  taken in 250 c.c. of water at 10.25 a.m. 2.5 litres of distilled water taken during the day. Curve A.—Mgms. Inorganic Phosphorus per 100 c.c. of Blood. Curve B.—Rate of Excretion of Phosphorus in the Urine in Mgms. per Hour. Curve C.—Volume of Urine in c.c.

corresponding "wet" day 1.85 grms. This slight difference is probably again due mainly to the improved absorption of the salt. The normal excretion of phosphorus is about 0.9 grms. per diem.

## 2. Partition of Ingested Phosphate between Plasma and Corpuscles.

As shown above, under normal conditions the concentrations of inorganic phosphate in plasma and corpuscles are identical. The following experiments

were done to see whether this equal partition obtains after the ingestion of phosphate. The dose taken was 7 grms. of the acid salt ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ).

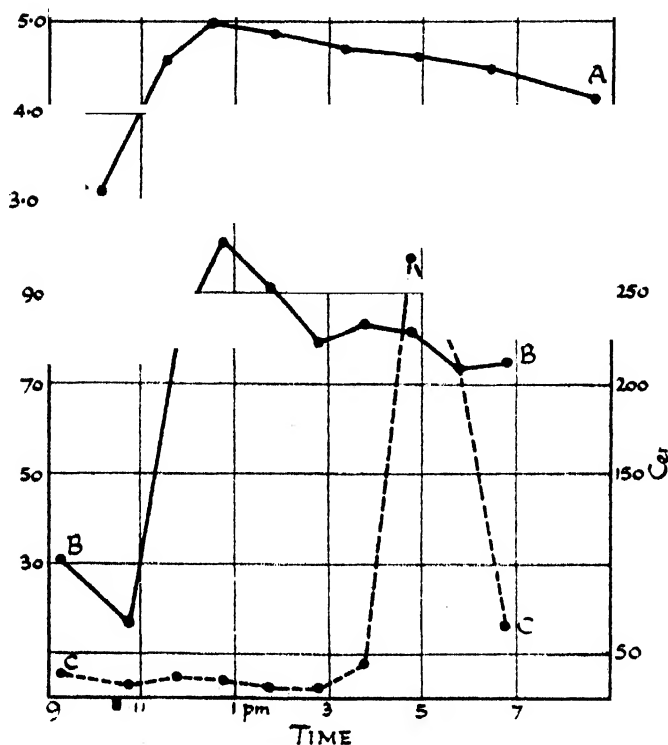


FIG. 4.—Subject C.E.W. 25 grms.  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  taken in 250 c.c. of water at 10.22 a.m. 2.5 litres of distilled water taken during the day. Curves A, B and C as in fig. 3.

Blood samples were obtained by venipuncture at intervals during the day. The inorganic phosphorus in the whole blood was estimated immediately. 5 c.c. of blood were centrifuged for 15 minutes and a second estimation carried out on 2 c.c. of plasma. Accurate hematocrit readings were not made, but the approximate proportions of plasma and corpuscles were obtained by means of a graduated centrifuge tube; and the distribution of phosphate between plasma and corpuscles was calculated on the basis of these figures. The results of two experiments are given in Tables II and III.

The phosphate of the plasma rises higher than that of the corpuscles, and remains so as long as the plasma value is high. As seen from the following



Table II.—Subject C.E.W. 7 grms.  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  taken at 10.20. Blood contained 42 per cent. corpuscles throughout the experiment.

Time .....	10.05	11.30	12.25	2.40	4.45	6.05
Inorganic P in whole blood in mgms. P per cent. ....	2.92	3.85	4.41	4.66	4.43	3.72*
Inorganic P in plasma in mgms. P per cent. ....	—	—	4.88	5.01	—	3.87*
Inorganic P in corpuscles (by calculation) .....	—	—	3.76	3.94	—	—

\* These values are the same within the error of the method.

Table III.—Subject V.B.W. 7 grms.  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  taken at 10.15. Blood contained 44.5 per cent. corpuscles throughout the experiment.

Time .....	10.00	12.20	3.15	6.20	10.00 a.m.
Inorganic P in whole blood in mgms. P per cent. ....	3.28	5.10	4.72	4.04	2.78
Inorganic P in plasma in mgms. P per cent. ....	3.16	5.61	5.18	4.37	2.73
Inorganic P in corpuscles (by calculation) .....	3.22	4.46	4.14	3.38	2.75

results (16), a similar relation holds when the phosphate concentration of the blood is raised far above normal by breathing an excess of carbon dioxide.

1. Plasma .. 5.07 .. Whole Blood 4.81.
2. Plasma .. 5.42 .. Whole Blood 5.04.

Thus, when the inorganic phosphate values are normal or sub-normal the values in plasma and corpuscles are identical within the limits of experimental error. When the values rise far above normal the plasma always contains more phosphorus than the corpuscles, no matter how the rise is produced. No difference was found between the values in whole blood for a given plasma value whether the phosphate content was rising or falling.

The explanation of these results is by no means clear. They cannot be explained by a slow diffusion of the  $\text{PO}_4$  ion through the corpuscular membranes, for in this case the plasma concentration would be expected to fall below that inside the corpuscles before the latter value had begun to fall. They are also equally difficult to explain on the view that there is no preformed phosphate in the corpuscles (Buell). It seemed possible that some of the inorganic phosphate after entering the corpuscle might be transformed into organic

compounds forming part of the "total acid soluble" fraction. Such a change has been demonstrated by Iversen (12) after the injection of very large doses of inorganic phosphate into rabbits. The following experiments do not show any change in the organic fraction of the "total acid soluble" phosphorus after taking 7 grms. of acid sodium phosphate. (Tables IV and V.)

Table IV.—Subject V.B.W. 7 grms. of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  taken at 10.30 a.m.

Time.....	10.20	12.40	2.40	6.10
Inorganic P. in mgms. P per cent. ....	3.31	5.45	4.81	3.50
Total acid soluble P in mgms. P per cent. ....	26.8	28.4	27.1	25.6
Organic acid soluble P (by difference)	23.5	23.0	22.3	22.1

Table V.—Subject C.E.W. 7 grms. of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  taken at 10.15 a.m.

Time.....	10.25	12.40	2.50	6.20
Inorganic P in mgms. P per cent. ....	3.36	5.08	4.87	3.55
Total acid soluble P in mgms. P per cent. ....	28.2	28.9	29.9	28.3
Organic acid soluble P (by difference)	24.8	23.8	25.0	24.7

The possibility still remains that the inorganic phosphorus has been converted into an organic form which is removed with the proteins.

The above experiments show that the acid soluble organic phosphorus is unaffected by phosphate ingestion. Reference may here be made to some experiments carried out to determine whether it could be changed by the ingestion of glucose. Since the view has been put forward by Robison (13) among others, that a hexose-phosphate forms a part of the acid soluble phosphorus, an increase might conceivably have been expected. The results given show, however, that after taking 100 grms. of glucose there is no measurable change in the organic acid soluble phosphorus of the blood.

Time.	Organic Acid Soluble P mgms. P/100 c.c. Blood.	Remarks.
11.05	22.4	100 grms. glucose.
11.15		
12.02	22.3	
1.20	21.8	

A further experiment was made when both an excess of glucose and phosphate were present in the blood simultaneously. The results are detailed in Table VI. Once again there is no change produced in the organic fraction of the acid soluble phosphorus.

Table VI.—Subject C.E.W. 7 grms. of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  were taken at 8.30; 100 grms. of Glucose at 9.45.

Time .....	8.15	10.35	1.30
Inorganic phosphate in blood in mgms. P per cent.	3.45	4.12	4.81
Total acid soluble phosphorus in mgms. P per cent.	28.5	28.8	29.0
Organic acid soluble phosphorus (by difference) .....	25.0	24.7	24.2

### *Discussion of Results.*

In the case of the dog, Denis (1) has shown that phosphate given either by the mouth or injected is excreted so rapidly by the kidney that there is no accumulation of the salt in the blood. In man we have found that the ingestion of much smaller amounts causes a 50 to 60 per cent. increase in the phosphate concentration of the blood, which only returns to the normal level very gradually. Apparently the human kidney, in the readiness with which phosphate is excreted, reacts differently to that of the dog.

The experiments throw some light on the relation between the concentration in the blood and the hourly excretion by the kidney. In our experiments the diurnal changes in phosphate output, and any variations due to the amount of acid to be excreted have, as far as possible, been obscured by the ingestion of large amounts of phosphate. Under these conditions the excretion by the kidney runs roughly parallel to the concentration in the blood. If the rate of excretion be plotted against the blood concentration, the points fall approximately about a straight line which, if continued, meets the axis at a concentration of phosphorus in the blood in the region of 2.4 mgms. per cent. If plasma values had been used, and all interfering factors had been entirely removed, then it might have been found that in its excretion phosphate behaves as a threshold body, whose excretion is proportional to the concentration above a threshold level in the blood. The diuresis produced by water drinking does not affect the rate of phosphate excretion appreciably; the output remains the same with a given concentration in the blood, while the urinary concentration varies from one-tenth to one-hundredth molecular.

The results which we have obtained for the distribution of phosphate between plasma and corpuscles with normal and abnormal concentrations of phosphorus in the blood are interesting on theoretical grounds. If the inorganic phosphate were all in solution in water we should expect the ratio of phosphorus to water in the corpuscles to be slightly less than that in the plasma. For their reaction, as shown by Warburg (14), is more acid; so, in accordance with Donnan's (15) principle, the ratio of phosphate to water should be less inside than outside, as is the case with chloride. Hence the phosphate per unit volume should be much less in corpuscles than in plasma. Our results would be explicable if some of the phosphate in the corpuscles were loosely held by adsorption or otherwise by the colloids; and if, when the concentration of phosphate rose above a certain value, this fraction increased less rapidly than the fraction dissolved in water.

#### *Summary.*

1. The ingestion of doses of the acid and alkaline sodium phosphates containing  $1\frac{1}{2}$ –2 grms. of phosphorus causes a 50–60 per cent. increase in the blood phosphate.
2. The curve of urinary excretion runs roughly parallel to that of the blood concentration, but the former varies much more widely than the latter, being roughly proportional to the excess above a certain value in the blood.
3. The rate of excretion of phosphate is independent of the amount of water excreted.
4. Under almost all the conditions investigated the concentrations of inorganic phosphate in plasma and corpuscles are identical.
5. When the concentration of phosphate in the plasma is far above normal, the value for the corpuscles is always lower whether the value in the plasma is rising or falling.
6. The organic fraction of the acid soluble phosphorus of the blood is not changed by the ingestion of phosphate, of glucose, nor of phosphate and glucose together.

We wish to express our thanks to Prof. Hopkins for the interest he has taken in this work, and to Mr. J. B. S. Haldane for much helpful criticism and advice. The expenses have in part been borne by grants to both of us from the Scientific and Industrial Research Board.

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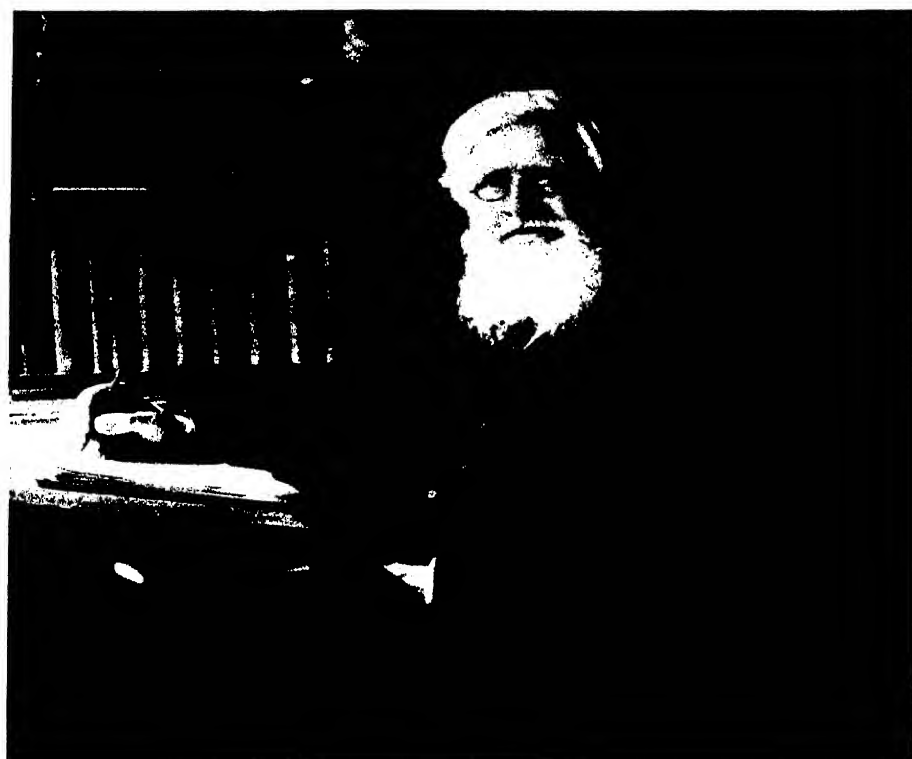
**OBITUARY NOTICES**  
**OF**  
**FELLOWS DECEASED.**

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## ALFRED RUSSEL WALLACE, 1823-1913.

LOOKING back on the life of Alfred Russel Wallace in this, the centenary of his birth, it is right to think of him as the last survivor of a band of comrades to whom we owe that growth in evolutionary thought which is probably the chief intellectual characteristic of the nineteenth century. Lyell, inspired by Buckland at Oxford, started the movement in his "Principles of Geology," of which Darwin said that it altered the whole tone of the reader's mind, so much so indeed that he felt, when he looked on any new geological feature, that he was seeing it with Lyell's eyes rather than his own. Then, in the onward rush, influencing and being influenced as Lyell was by his disciple, were Hooker, Huxley and Wallace. Nor must mention be omitted of H. W. Bates, whose friendship early in life was the determining cause of Wallace's journey to the tropics; nor of Herbert Spencer, a great power half a century ago, with his sonorous sentences and sublime infallibility. We remember how Darwin said that to read Spencer always made him feel like a worm, but that he retained the worm's privilege of wriggling, and at another time, more incisively, "wonderfully clever, and I dare say mostly true." And the story perhaps invented, but if so well invented, of Spencer's reply to an argument—"That can't be true, for otherwise 'First Principles' would have to be re-written—and the edition is stereotyped."

To create a true picture much must be said of Wallace's relations with nearly all these great men, and to do this demands a long notice, which will perhaps be excused because it deals with the chief actors in a critical epoch in the history of thought. It is a noteworthy fact that nearly all these men began their scientific career by long voyages or travels—Darwin, Hooker and Huxley with the Navy, Wallace and Bates in the South American tropics. With most of them, and especially with Wallace, the way to science was long and difficult. But this was not all loss: strength grows in one who

". . . grasps the skirts of happy chance  
And breasts the blows of circumstance  
And grapples with his evil star."

It should be remembered too that the want in those days of modern facilities which are only fair and right, prevented all but the most determined or the fortunate from following science. Scientific men were, therefore, comparatively few, and some among them reached a height above their fellows quite unattainable at this time when the general level of scientific achievement has been so immensely raised.

We are fortunate in the possession of materials enabling us to study the lives and thoughts of Lyell, Darwin, Huxley, Hooker and Wallace. We know, from his autobiography, what Wallace thought of his own career and the

influences which had shaped it, as he looked back at the age of 82, with a mind alert and vigorous as it had ever been. We know from the great store of his published letters what he thought at the time, and, from the letters of his comrades, what were their thoughts on the same subjects.

Many of Wallace's letters have appeared in more than one publication, and in quoting from these, reference will be made to the earliest dated volume except when a more complete copy is given in a later one. Slight verbal changes and alterations in punctuation have been occasionally made after comparing the original letters with the published version. Letters or passages quoted without reference are now published for the first time, and when the name of the correspondent is omitted that of the present writer is to be understood.

Wallace's father, Thomas Vere Wallace, a solicitor by training, lived until his marriage in 1807, upon the income of private property. Finding this insufficient he was persuaded to start an expensive magazine and thus lost a large part of his estate. By the time he had six children he was obliged to look out for a place where living was as cheap as possible. He fixed on a large cottage in Usk, Monmouthshire, where Alfred Russel was born, January 8, 1823,\* being thus fourteen years younger than Darwin. At Usk, Thomas Wallace kept down expenses by working in the large garden and by teaching his children himself. In 1828 he moved to Hertford and about ten years later to Hoddesdon, where he died in 1843. While at Hertford he was defrauded by a solicitor whom he had entrusted with the remainder of his property, and from this time to his death was reduced to the narrowest straits.

Alfred was the eighth of a family of four sons and five daughters. William, the second in the family, an architect and surveyor, who played an important part in his brother's early training and development, lived to be 36; John, the seventh child, 77; Herbert, the youngest, 22, when he died of yellow fever at Paris. Of the daughters three, the eldest, fifth and sixth of the family, died in infancy or childhood; the third as a young woman; while Frances (Mrs. Sims), the fourth, lived to be 81, the greatest age attained by any of the children except Alfred, who nearly completed his 91st year. Apart from Alfred Russel the only indications of unusual power appeared in the promising poetical and literary gifts of the youngest son.

\* The date 1822 will probably be found in some of the older records, but any uncertainty is dispelled by the following letter:—

“*February 23, 1903.*

“Up to about 15 years ago I thought I was born in 1822. I suppose I had been told so. But I then came into possession of an old Prayer Book in which the date of birth of my father is given by his father, and of all my brothers and sisters in my father's handwriting, and there I am put down as born in 1823, Jan. 8th, and the date is repeated for my baptism Feb. 16th, 1823. I therefore found myself then a year younger than I had supposed. . . .” (2, 468.)

The materials for arriving at a judgment are slender, but they lead us to infer that Wallace's qualities were chiefly inherited from his mother, a conclusion also suggested by the portraits. (4, I, 16 ; 6, I, 48.) He resembled his father, however, in being easily persuaded to risk and unfortunately to lose his property.

Wallace lived at Hertford between the ages of 6 and 14 and received his sole school education at the Grammar School. He left too early to begin Greek, but learnt enough French in 2-3 years to be able to read any easy book ; while six years of Latin produced the usual result—"a scanty knowledge of the vocabulary and grammar." Geography and history as then taught left painful memories, while mathematics was restricted to Euclid and algebra.

During his last year Wallace, probably in lieu of the school fees, taught reading, dictation, arithmetic and writing to the younger boys. But, being himself younger than many boys who did not teach, he constantly suffered from a feeling of humiliation, and for twenty years after leaving was troubled by dreams of his exceptional position in the school. Shyness, rendering him unable to refuse, was also the cause of the only occasions on which he took more wine than was good for him.

In many ways his education owed less to school than to his home-life, although it ended when he was fourteen, leaving him without a home until he started one of his own twenty-eight years later. In spite of its early close, Wallace always felt that the home of his childhood had made a very deep impression on his character. The fact that he was in no way influenced by the many disreputable young men with whom he was thrown into contact he attributed partly "to natural disposition, which was reflective and imaginative, but more perhaps to the quiet and order of my home, where I never heard a rude word or an offensive expression. The effect of this was intensified by my extreme shyness, which made it impossible for me 'to use words or discuss subjects which were altogether foreign to my home-life, as a result of which I have never been able to use an oath, although I have frequently felt those impulses and passions which in many people can only find adequate expression in such language." (4, I, 128.)

The separation effected by the gap of five years between him and John, the brother next above him in age, gradually disappeared and they became close companions, working and playing together in an old loft over the stable, making toys and fireworks and thus getting amusement and education out of their limited pocket-money. Wallace considered that the two or three years thus employed were certainly the most interesting and perhaps the most permanently useful of his whole boyhood. (4, I, 71.)

At home, too, he spent much time with books, of which a great variety was available, and enjoyed listening to his father, who read to the family in the evenings. He was brought up by religious but by no means narrow or rigid

parents as a member of the Church of England, but occasionally went to chapel, and it was there only that for a time he felt "something of religious fervour," chiefly inspired by the more picturesque and impassioned hymns. As, however, there was no sufficient basis of intelligible fact or connected reasoning to satisfy his intellect this feeling soon left him and never returned. (4, I, 78.) At 21 he was an agnostic, although in later life under other influences, mainly spiritualistic, his views became greatly modified.

He left school at Christmas, 1836, and early in the following year went to London and lived for a few months with his brother John, who was boarding with a small builder to whom he was apprenticed. He read Paine's "Age of Reason" and attended lectures on socialism and what was later on known as agnosticism, at a "Hall of Science." Under these influences he came to believe that "the only true and wholly beneficial religion was that which inculcated the service of humanity, and whose only dogma was the brotherhood of man." (4, I, 89.) His political views were founded on the teachings of Robert Owen and some of his disciples. He always regarded Owen as the founder of the Socialist movement in England.

Early in the summer of 1837 Wallace began to learn land-surveying in Bedfordshire, helping his brother William at Barton-in-the-Clay, six miles north of Luton. It was here that he first began to be interested in the features of the earth's surface, its plants and its geology, as well as in mapping and surveying. The methods adopted and the accurate results obtained in the trigonometrical survey of England inspired him to study the practical applications of mathematics, beginning with Mechanics and Optics. In the course of their survey at various places in the county they reached in 1838 Soulbury, on the canal, and here Wallace saw a steam-engine for the first time. A little later at Leighton Buzzard he and a tin-and-copper-smith with whom he lodged opened a tumulus near the town.

The surveying came to an end for a time and Wallace became shop-boy to a Mr. Matthews, and probably learned much by living nine months with one who combined occupations so diverse as surveying, watch and clock making, and the charge of the Leighton Buzzard gasworks.

In the autumn of 1839 Wallace recommenced land-surveying with his brother. Their work lay in Herefordshire and in the winter he went to correct an old map of the parish of New Radnor, where he stayed at the little inn and heard for the first time the story of Abélard and Héloïse from the district exciseman. After an illness following a chill contracted at Rhayader he soon found himself in the course of his work at Llandrindod Wells, surveying for the enclosure of common lands; but, at this time, no thought of land nationalization or even of the evils of enclosure had entered his mind. In the early summer of 1841 they went to Brecon to survey a parish near Trallong, where they stayed with an intelligent, well-educated, Welsh-speaking, shoemaker. Wallace

always made a point of visiting everything of interest or beauty in the neighbourhood of his work. Here it was the Beacons and a little later he was greatly impressed by his first sight of a megalithic stone, the "Maen Llia," nearly 12 feet high, at the head of the Llia River, a tributary of the Neath. Then, in the autumn of 1841, they began a six-months' survey of the extensive parish of Cadoxton-juxta-Neath, on the north side of the Neath valley, and here he had abundant opportunities of studying Welsh country life.

After Cadoxton came a change in the character of their work, bringing new experiences. They surveyed and sounded the three or four miles of the river between Neath bridge and the sea, obtaining data for navigation and docks. This work involved architecture and engineering for the building of warehouses and their appliances; it meant, too, the study with much advantage of Bartholomew's "Specifications for Practical Architecture."

During his stay in Neath, with plenty of time on his hands, Wallace laid the foundation of a deep interest in astronomy and botany. He practised simple observations and made his own telescope; he studied the plants found on lonely rambles over the hills and moors, and in 1841 bought his first simple book on botany, to be followed by Lindley's "Elements," costing 10s. 6d., a great sum for him in those days. In the margins of his Lindley, or on thin paper interleaves he copied from a borrowed Loudon's "Encyclopædia of Plants" the characters of every British species.

His brother looked upon these interests outside the profession as a waste of time, but Wallace, when he brought home a species of plant new to his herbarium, "experienced the joy which every discovery of a new form of life gives to the lover of nature, almost equal to those raptures . . . afterwards felt at every capture of new butterflies on the Amazon, or at the constant stream of new species of birds, beetles, and butterflies in Borneo, the Moluccas, and the Aru Islands." (4, I, 195.)

About this time his interest in orchids was first aroused by a nurseryman's catalogue at a flower-show at Swansea, where he first saw an epiphytic species of the group, and by an article by Lindley in the "Gardener's Chronicle." The enthusiasm thus aroused in him was, he believed, one of the causes which made him long for the tropics.

Wallace looked upon these professionally idle days at Neath as the turning-point of his life. He had always found surveying extremely interesting work, and, if there had been enough to do, he believed that he would not have sought any other occupation. He was evidently of an adaptable disposition, for he felt just the same about his next occupation, that of a schoolmaster (p. viii). In the spare time at Neath Wallace not only gave free play to his love for science, but also, in 1843, when he was between 20 and 21 years of age, began to write, preparing popular lectures on botany and on "The Advantages of Varied Knowledge," and an article on "The South Wales Farmer." It is interesting to notice, in this last (4, I, 206-222), his depreciatory remarks on

Welsh superstitions, and it may be supposed that some of his criticisms would have been softened had he written in later years.

His father died in April, 1843, and the family was broken up, the members seeking work of various kinds and in various directions. Shortly before his 21st birthday in January, 1844, his brother told him that he had no work for him and that he must find something for himself. At this point in his life, as he looked back upon it in his old age, he considered that his character was "fairly determined, although some portions of it had not yet had opportunity for full development." He "possessed a strong desire to know the causes of things, a great love of beauty in form and colour, and a considerable but not excessive desire for order and arrangement . . . If I had one distinct mental faculty more prominent than another, it was the power of correct reasoning from a review of the known facts in any case to the causes or laws which produced them, and also in detecting fallacies in the reasoning of other persons." In the use of his powers he was moved by "an intense appreciation of the beauty, harmony and variety in nature and in all natural phenomena, and an equally strong passion for justice as between man and man—an abhorrence of all tyranny, all compulsion, all unnecessary interference with the liberty of others." (4, I, 223, 224.)

He had no ear for music but "a fair appreciation of time, expression and general effect," and he was "deeply affected by grand, pathetic, or religious music." His power of drawing he considered very limited and his verbal memory and aptitude for learning foreign languages weak. Shyness, reticence and want of confidence he constantly mentions and evidently believed them to be extreme, although, as he said, "they have helped to give me those long periods, both at home and abroad, when, alone and surrounded only by wild nature and uncultured man, I could ponder at leisure on the various matters that interested me. Thus was induced a receptiveness of mind which enabled me at different times to utilize what appeared to me as sudden intuitions—flashes of light leading to a solution of some problem which was then before me . . ." He also speaks of "the total absence of wit or humour, paradox or brilliancy, in my writings, although no one can enjoy and admire these qualities more than I do." (4, I, 224-6.)

Unable to obtain further work in surveying, Wallace turned to the teaching profession, and after one fruitless application he became early in 1844 a master at the Collegiate School, Leicester, teaching English, reading, writing, and arithmetic to the junior classes, drawing to beginners and surveying to a few boys, also taking preparation. He was well treated and comfortable and had time to himself. The headmaster, the Rev. A. Hill, also kindly helped him in mathematics, and he studied algebra, trigonometry, the differential calculus, and reached, as he thought, in the integral calculus, the end of his powers. He subscribed to the town library and read, among many works, two which

profoundly influenced his future life. "Humboldt's *Personal Narrative of Travels in South America*," inspired him with the longing to see the tropics; Malthus' "Population," germinating in his mind, was to give him the clue to Natural Selection, as it had long since given the clue to Darwin, and Darwin's words in his autobiography show that Humboldt, read during his last year at Cambridge, had influenced him as strongly as it did Wallace. (3, I, 55.)

More important still, probably the most important event in his life, was the beginning of his friendship with Henry Walter Bates, then living at Leicester. Wallace believed that he first heard of Bates as an ardent entomologist and then met him at the town library. Bates introduced him to the fascinating study of British beetles—then quite unknown to him. "He asked me to see his collection, and I was amazed to find the great number and variety of beetles, their many strange forms and often beautiful markings or colouring, and was even more surprised when I found that almost all I saw had been collected around Leicester, and that there were still many more to be discovered." (4, I, 237.)

His interest thus aroused, Wallace bought Stephens' "Manual of British Beetles" and became an eager collector. When we remember the words used by Wallace at the 50th anniversary of the announcement of Natural Selection to the world, and how he spoke of beetle-collecting and all that it had meant to Darwin and himself \* we are made to realise the immense significance of the friendship with Bates in this one respect alone.

The time spent at Leicester also altered Wallace's whole outlook in other and very different ways. In 1844 he attended some lectures on mesmerism and was at once deeply impressed and began to try his powers upon boys in the school. The subject was evidently new to him and he looked upon it as his first introduction to psychical research. "The importance of these experiments to me was that they convinced me, once for all, that the antecedently incredible may nevertheless be true . . ." (4, I, 236.) Many of the effects he describes are well known to be obtainable in the hypnotic state and to be explained from the stand-point of our present knowledge, limited as it is, of the nervous system and its functions. But other effects, especially those believed to have been obtained by touching the "organs" of phrenology, belong to a different category. Having witnessed many genuine results that were "antecedently incredible" to him it is probable that he and others were thrown off their guard and thus rendered insufficiently critical of results and interpretations all the more seductive because they seemed to lead into certain bye-paths of the undeveloped and, in many ways, mistaken physiology of the day.

Wallace was much impressed but not convinced by the excellent extempore preaching to which he listened nearly every Sunday at Leicester, but his

\* Darwin-Wallace Celebration of the Linnean Society of London, p. 8 (1908).



"return to some form of religious belief was to come much later, and from a quite different source." (4, I, 240.)

Wallace was not only happy but evidently successful at the school. Outside his regular work, which was becoming more and more familiar to him, he wrote the rhymed prologue to be recited by a boy of 12 at one midsummer prize-giving, and a travesty of the story of Guy Faux as a play acted by the boys in 1845. All difficulties would have gradually disappeared and he might well have remained a schoolmaster all his life. The change was forced on him by the unexpected death of his brother William at Neath in February, 1845,\* and having made the break, his life became moulded by interests stirred at Leicester, outside the sphere of his professional work. "The events which formed a turning-point in my life were, first, my acquaintance with Bates, and through him deriving a taste for the wonders of insect-life, opening to me a new aspect of nature, and later on finding in him a companion without whom I might never have ventured on my journey to the Amazon. The other and equally important circumstance was my reading Malthus, without which work I should probably not have hit upon the theory of Natural Selection and obtained full credit for its independent discovery. My year spent at Leicester must, therefore, be considered as perhaps the most important in my early life." (4, I, 240.)

Having wound up his brother's affairs Wallace found that surveying was much in demand in consequence of the sudden rise of railway speculation. He therefore returned to his old profession and was at work all the summer of 1845, taking levels on the south-east side of the Neath Valley and going in the autumn to London in order to finish his plans, etc. Then, still finding plenty of work, he persuaded John to leave London and join him in Neath at the beginning of 1846, a year in which they surveyed the parish of Llantwit-juxta-Neath for tithe commutation, but the attempt to collect the payment from the farmers disgusted him with business and made him wish to give it up altogether. The brothers also designed buildings, of which the chief was the Mechanics' Institute at Neath, and Alfred lectured to the mechanics of the town in the two winters of their residence.

\* The year is given as 1846 in Wallace's Life (4, I, 239) and in the shortened edition (5, 129), but as 1845 in data kindly supplied by Mr. W. G. Wallace. The year 1846 would make the critical period at Leicester two years instead of one or a little over (4, I, 230, 240); for Wallace joined the school staff early in 1844 and left at Easter in the year of his brother's death. The inconsistency was evidently observed by Mr. W. G. Wallace in preparing the shortened Life, for the period is there said to be two years. (5, 130.) Mr. Wallace now agrees with me that the error is in the date given for his uncle's death, and that the period at Leicester was a little over one year. This conclusion is confirmed by the statement that John joined his brother in the January following William's death and that they lived together at Neath in the two summers before April, 1848, when Wallace sailed for Pará. (4, I, 244, 247, 262; 5, 133, 135, 145.)

At this time too, his belief in phrenology was strengthened by two estimates of his character by itinerant lecturers. He corresponded with Bates, who spent a week with them in the summer probably of 1847, and Wallace believed that it was during this visit that they discussed the plan of a journey to the tropics together.

A letter to Bates, April 11, 1846, shows that Wallace was influenced by Darwin's "Journal" and by Lyell, who had influenced Darwin so profoundly—"I was much pleased to find that you so well appreciated Lyell. I first read Darwin's 'Journal' three or four years ago, and have lately re-read it. As the Journal of a scientific traveller, it is second only to Humboldt's 'Personal Narrative'—as a work of general interest, perhaps superior to it. He is an ardent admirer and most able supporter of Mr. Lyell's views. His style of writing I very much admire, so free from all labour, affectation, or egotism, and yet so full of interest and original thought." (4, I, 256.) Thinking over these words, half a century later, Wallace believed that they indicated the two works to whose inspiration he owed his determination to visit the tropics. (*Loc. cit.*)

As early as 1847 Wallace was thinking about the origin of species, reading the "Vestiges" and writing about this book to his friend Bates. "I have rather a more favourable opinion of the 'Vestiges' than you appear to have," he wrote from Neath on December 28; "I do not consider it a hasty generalisation, but rather an ingenious hypothesis strongly supported by some striking facts and analogies, but which remains to be proved by more facts and the additional light which more research may throw upon the problem. It furnishes a subject for every observer of nature to attend to; every fact he observes will make either for or against it, and it thus serves both as an incitement to the collection of facts, and an object to which they can be applied when collected. Many eminent writers support the theory of the progressive development of animals and plants." And he then goes on to speak of Lawrence's "Lectures on Man," maintaining that both he and Prichard in his "Physical History of Man" concluded "that the varieties of the human race have not been produced by any external causes, but are due to the development of certain distinctive peculiarities in some individuals which have thereafter become propagated through an entire race." (4, I, 254-5.)

It seems probable from this reference to Prichard's great work that Wallace had read the 2nd edition, 1826, containing an anticipation of some modern views on evolution (p. xxiii).

In 1847 he wrote to Bates, after a day in the Insect-room of the British Museum :—

"I begin to feel rather dissatisfied with a mere local collection; little is to be learnt by it. I should like to take one family to study thoroughly, principally with a view to the theory of the origin of species. By that means I am strongly of opinion that some definite results might be arrived at." (4, I, 256-7.) And the same haunting problem was before him when, at the end of the letter, he

wrote: "There is a work published by the Ray Society I should much like to see, Oken's '*Elements of Physiophilosophy*.' . . . It contains some remarkable views on my favourite subject—the variations, arrangements, distribution, etc., of species." (*Loc. cit.*)

A visit to the tropics having been decided upon, W. H. Edwards' "*Voyage up the Amazon*," published in 1847, and read in that year or very early in 1848, was the determining cause for the selection of the mighty river by Wallace and his friend. They arranged with Samuel Stevens to act as their London agent for the care and disposal of collections, and sailed from Liverpool on April 20, 1848, arriving at Pará twenty-nine days later. The wealth of insect life awaiting them may be inferred from the fact that over 400 species of butterflies were collected in about two months.

The fact that Wallace and Bates soon separated and explored different parts of the Amazon Valley has been sometimes ascribed to disagreement or friction, and Wallace's views on spiritualism have been mentioned as a cause of difference between them. There are no grounds for these conclusions. Wallace did not begin to be interested in spiritualism until many years later, after his return from the East, and then Bates was one of the friends who encouraged him to investigate the subject. Furthermore, their correspondence during Bates' residence on the Upper Amazon and their immediate and most friendly association, after they had returned to England, are entirely consistent with Wallace's account of the reasons for their decision to separate:—

"For the first four months Bates and I lived and collected together in and around Pará, but on our return from an expedition which we had made up the Tocantins River, we agreed that it would be better for many reasons to travel and collect independently; one reason being that the country was so vast and so rich in birds and insects that much better results would be obtained if we each explored separate districts. We therefore separated, but we again met at Santarem and at the Barra. Afterwards Bates devoted himself to the Upper Amazon, while I ascended the Rio Negro and the unknown Uaupés." (5, 146.)

Mr. William G. Wallace informs me that he never heard of any disagreement between his father and Bates and he adds that, in addition to the reasons given in the above-quoted passage, "it is probable that they had to consider the commercial aspect of collecting. They could not expect to get such good prices if they merely sent home the same material. I think there is no doubt, too, that there was more opportunity for earning scientific distinction by working independently, and this may have influenced two keen young naturalists." Leaving Pará, Wallace went up the Amazon to Santarem, near the mouth of the Tapajos, a southern tributary, and here for the first time observed the separation of the areas occupied by two representative species of butterflies by a river barrier. From Santarem he went much higher up the Amazon to

Barra, the modern Manaos, at the mouth of the Rio Negro, a northern tributary. After some months near Barra, which he found very poor in insect and bird life—and indeed no other locality visited by him was at all equal to Pará—Wallace prepared for the first of his two expeditions, in 1851 and 1852, up the Rio Negro, while his brother Herbert, who had joined him, returned to Pará, where he died on the eve of his departure for home. On his first expedition Wallace travelled beyond the Brazilian boundary and crossed the divide by a forest road to a tributary of the Orinoco, also travelling up the Uaupés, a tributary of the Negro, as far as the second cataract. On his second journey, when he went much further up the Uaupés, he suffered so terribly from fever and dysentery that he was compelled to return home. At one time he was so ill that he was not expected to live through the night. In his later travels in the Malay Archipelago, Wallace was able to subdue fever with quinine, of which he considered that he did not take half enough in South America.

On these expeditions, Wallace made drawings of about 200 species of fishes, many of which remain undescribed at the present day. His four years in the Amazon Valley left the following dominant impressions:—

First and foremost he placed the virgin forest, that same tropical American forest which had impressed W. J. Burchell and Darwin (3, I, 237) more than anything in their travels. From a scientific point of view the fact which struck him most was the number of the species of forest trees in a limited area. On his later journey in the Malay Archipelago he estimated that there were 1,500 species in the forested areas of Java, which, taken together were, he considered, only a little larger than Wales.

The second impression which stood out in his memory was the variety and beauty of the birds and butterflies.

“The third and most unexpected sensation of surprise and delight was my first meeting and living with man in a state of nature—with absolute uncontaminated savages! This was on the Uaupés river, and the surprise of it was that I did not in the least expect to be so surprised. I had already been two years in the country, always among Indians of many tribes; but these were all what are called tame Indians, they wore at least trousers and shirt . . . .

“But these true wild Indians of the Uaupés were at once seen to be something totally different. They had nothing that we call clothes; they had peculiar ornaments, tribal marks, etc.; they all carried weapons or tools of their own manufacture; they were living in a large house, many families together, quite unlike the hut of the tame Indians; but, more than all, their whole aspect and manner were different—they were all going about their own work or pleasure which had nothing to do with white men or their ways; they walked with the free step of the independent forest-dweller, and, except the few that were known to my companion, paid no attention whatever to us, mere strangers of an alien race. In every detail they were original and self-sustaining as are

the wild animals of the forests, absolutely independent of civilization, and who could and did live their own lives in their own way, as they had done for countless generations before America was discovered. I could not have believed that there would be so much difference in the aspect of the same people in their native state and when living under European supervision. The true denizen of the Amazonian forests, like the forest itself, is unique and not to be forgotten." (4, I, 288.)

Wallace sailed from Pará on July 12, 1852, and on August 6 it was found that a fire which could not be subdued was raging in the ship, a disaster due to the captain's ignorance of balsam-capivi and its dangers as a cargo, and his folly in opening the hatchways to pour in water and thus allowing air to gain access to the heated mass below. The ship was abandoned, and ten days and nights were spent in open boats in the attempt to traverse the 700 miles between the wreck and the Bermudas. When, however, they had sailed 500 miles they were taken on board the *Jordeson*, a slow and rotten sailing ship bound for London, which was reached after a miserable voyage on October 5. Wallace had intended to send his collections to England by an earlier boat, but unfortunately they were detained by the customs regulations at Barra, and everything except his drawings of fishes\* and palms was destroyed in the fire. Mr. Stevens had wisely insured them, enabling Wallace to recover £200.

By Christmas, 1852, Wallace was settled in London, at 44, Upper Albany Street, which he had taken in order that his mother and sister and her husband might live with him. Here he wrote papers for the scientific societies on his zoological and geographical discoveries, and also published a small volume on the "Palm Trees of the Amazon" and his "Travels on the Amazon and Rio Negro." At an evening meeting of the Zoological Society in December, 1852, he saw Huxley for the first time. Huxley was reading a paper on the Echinococci found in the liver of the zebra, and Wallace was immensely impressed by his knowledge and power of making the subject clear and interesting. Many years later he was surprised to find that Huxley was two years younger than himself.

Wallace soon began to think of another journey to the tropics. He was satisfied by his work at the Scientific Societies and at the British Museum that the Malay Archipelago offered the finest field for his future exploration, while his former experience enabled him to make far more complete preparations for determining the known species and for deciding whether those unknown to him were new to science.

Mr. Samuel Stevens again acted as his agent, and I have been told by Prof. Westwood that Mr. Wilson Saunders, who was then building up his great collection of insects, offered Wallace a shilling each for male and female—it

\* The drawings of fishes are now in the Zoological Library of the Natural History Museum. "Proc. Zool. Soc. Lond.," vol. I, p. 189 (1905).

may have been two males and two females—of every species he could take in the Malay Archipelago. This scale of payment does not seem to be high, but the number of species was so great that Wallace received a large sum, and the Wallace specimens are the great feature of the various sections of the Saunders Collection, now scattered in different directions.

Wallace started early in 1854, being granted, through the influence of Sir Roderick Murchison, a passage in a P. & O. steamer to Singapore. The passengers landed, as was customary in those days, at Alexandria, went by boat to Cairo and thence to Suez in small two-wheeled, four-horsed omnibuses; goods, mails and luggage being carried by camels. At Suez he went on board a very comfortable vessel in which he travelled to Singapore “where,” in his own words, “I was to begin the eight years of wandering throughout the Malay Archipelago, which constituted the central and controlling incident of my life.” (4, I, 336.)

After collecting in Singapore and Malacca he went in November, 1854, to Sarawak, Borneo, where he remained fourteen months, receiving much kindness and hospitality from Sir James Brooke, the first Rajah of Sarawak, for whom he had a great admiration. In this period he wrote to his sister, Mrs. Sims, about the qualifications of a young assistant he hoped to employ. His letter suggests a picture of the conditions in those days, showing at the same time his own supreme equipment as a traveller. He wrote June 25, 1855, from the Sadong River, Borneo:—

“Let me know what you think of him. Do not tell me merely that he is ‘a very nice young man.’ Of course he is. . . . I have written to Mr. Stevens to let me know his character, as regards *neatness* and *perseverance* in doing anything he is set about. From you I should like to know whether he is quiet or boisterous, forward or shy, talkative or silent, sensible or frivolous, delicate or strong. Ask him whether he can live on rice and salt fish for a week on an occasion—whether he can do without wine or beer, and sometimes without tea, coffee or sugar—whether he can sleep on a board—whether he likes the hottest weather in England—whether he is too delicate to skin a stinking animal—whether he can walk twenty miles a day—whether he can work, for there is sometimes as hard work in collecting as in anything. Can he draw (not copy)? Can he speak French? Does he write a good hand? Can he make anything? Can he saw a piece of board straight? . . . Ask him to make you anything—a little card box, a wooden peg or bottle-stopper, and see if he makes them neat, straight and square.” (6, I, 57, 58.)

This letter was written towards the beginning and may well be read with another written towards the end of his journeys. Emphasising by contrast the comfort of Java he wrote to his mother July 20, 1861, from Sourabaya:—

“I shall no more be obliged to carry about with me that miscellaneous lot of household furniture—bed, blankets, pots, kettles and frying pan, plates,

dishes and wash-basin, coffee-pots and coffee, tea, sugar and butter, salt, pickles, rice, bread and wine, pepper and curry powder, and half a hundred more odds and ends, the constant looking after which, packing and repacking, calculating and contriving, have been the standing plague of my life for the last seven years. You will better understand this when I tell you that I have made in that time about eighty movements, averaging one a month, at every one of which all of these articles have had to be re-arranged and repacked by myself according to the length of the trip, besides a constant personal supervision to prevent waste or destruction of stores in places where it is impossible to supply them." (6, I, 84.)

It is unnecessary to repeat what has been told so fully and so well of Wallace's travels in the East, but some of the thoughts which came to him in that stirring time which produced the Joint Essay on Natural Selection and the 'Origin,' thoughts often expressed in letters written at the time, compel our attention.

The important paper "On the Law which has regulated the Introduction of New Species,"\* dated February, 1855, was written during the Bornean wet season, while he was staying in a little house at the mouth of the Sarawak River, at the foot of the Santubong mountain. (4, I, 354.) The law was thus stated:—"Every species has come into existence coincident both in time and space with a pre-existing closely-allied species," and this, as he justly claimed, "connects together and renders intelligible a vast number of independent and hitherto unexplained facts." This paper, the outcome of quiet thought, in evenings and wet days, on the distribution of animals, was the "powerful essay" of which Huxley wrote—"On reading it afresh, I have been astonished to recollect how small was the impression it made" (3, II, 185; 4, I, 355), and Darwin, writing to the author—"I agree to the truth of almost every word." (3, II, 95.) It is interesting to learn that the same comment was uttered then that has been made so often since upon almost every attempt to explore the processes of organic evolution; for Wallace remembered that "soon after this article appeared, Mr. Stevens wrote me that he had heard several naturalists express regret that I was 'theorizing,' when what we had to do was to collect more facts." (4, I, 355.)

It is of special interest to read the impressions of Bates when he received a copy of the paper from his old travelling companion. His letter was written, November 19, 1856, from Tunantins on the Upper Amazon:—

" . . . I was startled at first to see you already ripe for the enunciation of the theory. You can imagine with what interest I read and studied it, and I must say that it is perfectly well done. The idea is like truth itself, so simple and obvious that those who read and understand it will be struck by its

\* "Ann. Mag. Nat. Hist.," vol. XVI, 2nd ser., p. 184 (1855).

simplicity ; and yet it is perfectly original. The reasoning is close and clear, and although so brief an essay, it is quite complete, embraces the whole difficulty, and anticipates and annihilates all objections.

" Few men will be in a condition to comprehend and appreciate the paper, but it will infallibly create for you a high and sound reputation. The theory I quite assent to, and, you know, was conceived by me also, but I profess that I could not have propounded it with so much force and completeness." (6, I, 64-5.)

Bates then went on to speak of the work which the theory would inspire, especially the " noble subject " of special study in some group peculiar to a region " but offering different species in each province of it—tracing the laws which connect together the modifications of forms and colour with the *local* circumstances of a province or station—tracing as far as possible the actual *affiliation* of the species " (*loc. cit.*) : and he instanced the Heliconiid butterflies and Erotylid beetles of South America, preferring the latter because more local, although he finally chose the former for his classical monograph.

Wallace wrote, January 4, 1858, from Amboyna, in the Moluccas, in reply to his friend, who was then at St. Paulo, on the Upper Amazon, but left for Ega within a month :—

" To persons who have not thought much on the subject I fear my paper on the ' Succession of Species ' will not appear so clear as it does to you. That paper is, of course, merely the announcement of a theory, not its development. I have prepared the plan and written portions of a work embracing the whole subject, and have endeavoured to prove in detail what I have as yet only indicated. It was the promulgation of Forbes's theory of ' polarity ' which led me to write and publish, for I was annoyed to see such an ideal absurdity put forth, when such a simple hypothesis will explain all the facts. I have been much gratified by a letter from Darwin, in which he says that he agrees with ' almost every word ' of my paper. He is now preparing his great work on ' Species and Varieties,' for which he has been collecting materials twenty years. He may save me the trouble of writing more on my hypothesis, by proving that there is no difference in nature between the origin of species and of varieties ; or he may give me trouble by arriving at another conclusion ; but, at all events, his facts will be given for me to work upon. Your collections and my own will furnish most valuable material to illustrate and prove the universal applicability of the hypothesis. The connection between the succession of affinities and the geographical distribution of a group, worked out species by species, has never yet been shown as we shall be able to show it." (4, I, 358.)

In the same letter to Bates he spoke of the boundary since known as " Wallace's Line," passing between Bali and Lombok, and between Borneo and Celebes—a discovery due to the failure to find a vessel sailing direct from Borneo to Macassar. He therefore went to Lombok and studied both it



and Bali for two and a half months, while he waited for a ship. The classical discovery is thus described in the letter :—

“In this archipelago there are two distinct faunas rigidly circumscribed, which differ as much as do those of Africa and South America, and more than those of Europe and North America ; yet there is nothing on the map or on the face of the islands to mark their limits. The boundary line passes between islands closer together than others belonging to the same group. I believe the western part to be a separated portion of continental Asia, while the eastern is a fragmentary prolongation of a former west Pacific continent. In mammalia and birds the distinction is marked by genera, families, and even orders confined to one region ; in insects by a number of genera, and little groups of peculiar species, the families of insects having generally a very wide or universal distribution.” (4, I, 358-9.)

The fruitful delay in Lombok was followed, not many months later, by one still more fruitful in Ternate, when the idea of Natural Selection came to him while he was waiting and making preparations for the next journey.

Although the letter to Bates (p. xv) shows that Wallace was at the time without any solution of the great problem of species, “yet,” in his own words, “less than two months later that solution flashed upon me, and to a large extent marked out a different line of work from that which I had up to this time anticipated.” (4, I, 359.)

“In other parts of this letter I refer to the work I hoped to do myself in describing, cataloguing, and working out the distribution of my insects. I had in fact been bitten by the passion for species and their description, and if neither Darwin nor myself had hit upon ‘Natural Selection,’ I might have spent the best years of my life in this comparatively profitless work. But the new ideas swept all this away. I have for the most part left others to describe my discoveries, and have devoted myself to the great generalizations which the laborious work of species-describers had rendered possible.” (4, I, 359-60.)

“My paper written at Sarawak rendered it certain to my mind that the change had taken place by natural succession and descent—one species becoming changed either slowly or rapidly into another. But the exact process of the change and the causes which led to it were absolutely unknown and appeared almost inconceivable.” (*Loc. cit.*)

The difficulty he felt was the fact that species were so distinct and not an inextricable tangle of transitional forms, and that the distinctions are manifest in their food, the places they frequent, their instincts and habits, no less than in their visible characters. Furthermore, the characters of a species taken together are not a mere random assortment of differences from other species, but are held together by the fact that they are adapted to distinct conditions and modes of life. These difficulties are much the same as those felt by Darwin and spoken of in his Autobiography (3, I, 82) and other writings. With these

insistent difficulties in their minds we can well understand both the trend towards Natural Selection and the immense relief when once it had flashed upon them.

It is unnecessary to speak here of the oft-repeated and never-to-be-forgotten story of the Ternate paper sent to Darwin and the joint essay of July 1, 1858, bringing Natural Selection before the world. But passages from one of the letters which Wallace wrote when he received a copy of the "*Origin*" cannot be omitted. Although we know that the book was sent to him some days before its publication on November 24, 1859, it is a surprise to learn that Wallace had made the time to read it "five or six times" by the first of the following September. On this day he wrote from Bessir, a little village on the south coast of Waigiu, in the Moluccas, to his life-long friend, George Silk :—

" . . . The other book you may have heard of and perhaps read, but it is not one perusal which will enable any man to appreciate it. I have read it through five or six times, each time with increasing admiration. It will live as long as the '*Principia*' of Newton. . . . Mr. Darwin has given the world a *new science*, and his name should, in my opinion, stand above that of every philosopher of ancient or modern times. The force of admiration can no further go !!!" (4, I, 372-3.)

Wallace was back again in Ternate when he wrote, December 24, 1860, to Bates, who had returned from the Amazon :—

" I know not how, or to whom, to express fully my admiration of Darwin's book. To *him* it would seem flattery, to others self-praise ; but I do honestly believe that with however much patience I had worked and experimented on the subject, I could *never have approached* the completeness of his book, its vast accumulation of evidence, its overwhelming argument, and its admirable tone and spirit. I really feel thankful that it has *not* been left to me to give the theory to the world. Mr. Darwin has created a new science and a new philosophy ; and I believe that never has such a complete illustration of a new branch of human knowledge been due to the labours and researches of a single man. Never have such vast masses of widely scattered and hitherto quite unconnected facts been combined into a system and brought to bear upon the establishment of such a grand and new and simple philosophy." (4, I, 374.)

A third letter, now in the care of the Trustees of the British Museum, was written, from a somewhat different point of view, to his brother-in-law, Mr. T. Sims, who had evidently criticised the "*Origin*." It is dated March 15, 1861, from Delli, in the island of Timor :—

" It was only on the *fifth* perusal that I fully appreciated the whole strength of the work, and as I had been long before familiar with the same subjects I cannot but think that persons less familiar with them cannot have any clear idea of the accumulated argument by a single perusal.

" . . . It seems to me . . . as clear as daylight that the principle of Natural Selection *must* act in nature. It is almost as necessary a truth

as any of mathematics. . . . It is the vast *chaos* of facts, which are explicable and fall into beautiful order on the one theory, which are inexplicable and remain a chaos on the other, which I think must ultimately force Darwin's views on any and every reflecting mind. Isolated difficulties and objections are nothing against this vast cumulative argument. The human mind cannot go on for ever accumulating facts which remain unconnected and without any mutual bearing and bound together by no law." And then, anticipating Huxley's argument,\* he continued :—"The evidence for the production of the organic world by the simple laws of inheritance is exactly of the same nature as that for the production of the present surface of the earth . . . by the slow and natural action of natural causes now in operation. The mind that will ultimately reject Darwin must (to be consistent) reject Lyell also." (6, I, 77-8.)

He concludes that Darwin's larger work will not add to the strength of his argument but that "the smaller work will remain for general purposes the best. . . ." thus agreeing with Darwin himself, who wrote long afterwards in his Autobiography, "It is no doubt the chief work of my life." (3, I, 86.)

In addition to these letters and his defence of Darwin in books published at a later date, reference must be made to the letter in "Nature" of November 17, 1870, which so greatly pleased Darwin. (4, II, 7.)

A letter to his sister, Mrs. Sims, dated October 10, 1861, "In the mountains of Java," proves that the views expressed in his publications on tropical nature were present and fully formed in his mind during his travels. Writing of Java, which he looked upon as "the Garden of the East, and probably without any exception the finest island in the world" (6, I, 83), he nevertheless compared it unfavourably with his memories of English scenery and plant-life.

"This region exhibits all the beauty the tropics can produce, but still I consider and will always maintain that our own meadows and woods and mountains are more beautiful. Our own weeds and wayside flowers are far prettier and more varied than those of the tropics. It is only the great leaves and the curious-looking plants, and the deep gloom of the forests and the mass of tangled vegetation that astonish and delight Europeans, and it is certainly grand and interesting and in a certain sense beautiful, but not the calm, sweet, warm beauty of our own fields, and there is none of the brightness of our own flowers; a field of buttercups, a hill of gorse or of heather, a bank of foxgloves and a hedge of wild roses and purple vetches surpass in *beauty* anything I have ever seen in the tropics. This is a favourite subject with me. . . ." (6, I, 86-7.)

\* "The origin of a new species by other than ordinary agencies would be a vastly greater 'catastrophe' than any of those which Lyell successfully eliminated from sober geological speculation." (3, II, 190.)

Wallace had ample time during his travels for reflection on subjects outside the range of his favourite science. The Timor letter to his brother-in-law, from which I have already quoted (p. xvii), is followed by an interesting post-script on his religious views. He here argues that belief is quite independent of the will and against the view that it is "*voluntary*" and also that it is *meritorious*." After arguing in favour of this at considerable length he proceeded to apply the principle to his own experience.

"In my early youth I heard, as ninety-nine-hundredths of the world do, only the evidence on one side, and became impressed with a veneration for religion which has left some traces even to this day. . . I spent, as you know, a year and a half in a clergyman's family and heard almost every Tuesday the very best, most earnest and most impressive preacher it has ever been my fortune to meet with, but it produced no effect whatever on my mind. I have since wandered among men of many races and many religions. I have studied man, and nature in all its aspects, and I have sought after truth. In my solitude I have pondered much on the incomprehensible subjects of space, eternity, life and death. I think I have fairly heard and fairly weighed the evidence on both sides, and I remain an *utter disbeliever* in almost all that you consider the most sacred truths. I will pass over as utterly contemptible the off-repeated accusation that sceptics shut out evidence because they will not be governed by the morality of Christianity. You I know will not believe that in my case, and I know its falsehood as a general rule. I only ask, Do you think I can change the self-formed convictions of twenty-five years, and could you think such a change would have anything in it to merit *reward from justice*? I am thankful I can see much to admire in all religions. To the mass of mankind religion of some kind is a necessity. But whether there be a God and whatever be His nature; whether we have an immortal soul or not, or whatever may be our state after death, I can have no fear of having to suffer for the study of nature and the search for truth, or believe that those will be better off in a future state who have lived in the belief of doctrines inculcated from childhood, and which are to them rather a matter of blind faith than intelligent conviction." (6, I, 81-3.)

A characteristic note is added—"This for yourself; show the *letter only* to my mother." And the same affectionate consideration for her feelings appears in words written from Java, July 21, 1861, when the return home was filling his thoughts:—"Of course, my dear mother, I should not think of living anywhere but with you, after such a long absence, if you feel yourself equal to house-keeping for us both. . . ." (6, I, 84.)

There can be no doubt that Wallace at first contemplated systematic work in Zoology on a large scale and dealing himself with the material he was collecting. Thus he wrote, March 2, 1858, from Ternate, to F. Bates, the brother of his old friend:—"What would be the use of accumulating materials which

one could not have time to work up?" and giving this as a reason for not remaining for many years in the Archipelago. (6, I, 70.) But after returning to England and doing excellent work in systematics he soon began to feel that there were many other men who could do this better than he could, while his special tastes led him to researches which involved a good deal of reasoning and generalisation. (4, II, 94.) Bates, on the other hand, surprising many of his friends, after his classical paper on Mimicry, devoted nearly all the time he could spare to the Systematics of certain groups of beetles. The probable explanation is that, after his labours as a most capable and energetic Secretary of the Geographical Society, he could not attempt anything involving severe mental effort. His descriptive work was of high value in itself and imposed no intolerable strain.

Wallace reached home in the spring of 1862 and took a house in London where he lived for several years, writing papers on his collections and observations—on the birds and insects, the physical geography and the human races of the Malay Archipelago. This work, fascinating as it was, opened his eyes to the fact that he might spend the rest of his life describing his collections and still be far from completing the task. He therefore began to disperse his private collections to specialists in the various groups, and in 1867 and 1868 wrote the great book on his travels—the "*Malay Archipelago*."

He married in 1866 the daughter of William Mitten, the authority on Mosses. Their friends will always remember the great love for plants which Wallace and his wife had in common, a love which brought a constantly renewed and eager happiness into his life.

He had written, from Singapore, on the eve of his return home, to his intimate friend, George Silk, on the subject of marriage:—"I believe a good wife to be the greatest blessing a man can enjoy, and the only road to happiness, but the qualifications I should look for are probably not such as would satisfy you. My opinions have changed much on this point: I now look at intellectual companionship as quite a secondary matter, and should my good stars ever send me an affectionate, good-tempered and domestic wife, I shall care not one iota for accomplishments or even for education." (6, I, 87-8.)

Friends who have had the privilege of visiting his home, who have witnessed the love for plants and animals shared by Wallace and his wife, have seen the plants reared with loving care by her, and the robin in the garden flying to her hand for food, will know how imperfectly these earlier views anticipated the happy companionship that was to be his.

Wallace applied for various posts—the Secretaryship of the Royal Geographical Society, which Bates held for so many years, the Directorship of the Bethnal Green Museum, and the position of Superintendent of Epping Forest—but on the whole considered it an advantage that he was unsuccessful; for any of them would have taken from him the freedom and quiet without which he could not have written.

To this period of his life belongs one of the most interesting episodes described by Wallace—the call paid by him and Bates upon Herbert Spencer. The two enthusiastic naturalists soon after their return home from the tropics had been immensely impressed by “*First Principles*.” This “wonderful exposition of the fundamental laws and conditions, actions and interactions of the material universe seemed to penetrate so deeply into that ‘nature of things’ after which the early philosophers searched in vain and whose blind gropings are so finely expressed in the grand poem of Lucretius, that we both hoped he could throw some light on that great problem of problems”—the origin of life. “I think Bates was the chief spokesman, and expressed our immense admiration of his work, and that as young students of nature we wished to have the honour of his acquaintance. He was very pleasant, spoke appreciatively of what we had both done for the practical exposition of evolution, and hoped we would continue to work at the subject. But when we ventured to touch upon the great problem, and whether he had arrived at even one of the first steps towards its solution, our hopes were dashed at once. That, he said, was too fundamental a problem to even think of solving at present. . . . All he could say was that everything pointed to its having been a development out of matter—a phase of that continuous process of evolution by which the whole universe had been brought to its present condition.” The story of the interview ends with the characteristic statement, “And now, after forty years, . . . whatever light we do possess is from a source which Spencer and Darwin neglected or ignored.” (4, II, 23, 24.)

It is also of much interest to learn that Herbert Spencer strongly urged Wallace to attack the late Lord Salisbury’s 1894 address to the British Association at Oxford.

Finally, after speaking of the differences between himself and Spencer, Wallace concludes:—“I yet look upon these as but spots on the sun of his great intellectual powers, and feel it to be an honour to have been his contemporary, and, to a limited extent, his friend and coadjutor.” (4, II, 33.)

The same charming modesty and the same power—sometimes wanting in great minds—of recognising and admiring the greatness of others, appear again and again in Wallace’s memories, and I well remember his surprise at Spencer’s letter acknowledging the gift of a copy of “*Darwinism*.” About six months after receiving the letter, while staying with us at Oxford, he repeated the substance of the words written by Spencer on May 18, 1889:—

“I regret that you have used the title ‘*Darwinism*,’ for notwithstanding your qualification of its meaning you will, by using it, tend greatly to confirm the erroneous conception almost universally current.” (6, II, 47.)

During the same visit, when he received the Hon. D.C.L. degree, I was anxious to show him something of Oxford; but, with all that there is to be seen, one subject alone absorbed the whole of his interest—he was intensely anxious to find the rooms where Grant Allen had lived. He had received from Grant

Allen's father a manuscript poem giving a picture of the ancient city dimly seen at midnight from an undergraduate's rooms. With the help of Grant Allen's college friends we were able to visit every house in which he had lived, but were forced to believe that the poem was written in the rooms of a friend or from an imaginary point of view. (1, 348; 6, II, 219.) The latter turned out to be the solution, for Grant Allen can hardly have actually been "On Magdalen Tower" when he wrote the poem published\* with this title, as I am kindly informed by Mr. Edward Clodd.

Between the years 1863 and 1872, when he was living in London, Wallace saw more of Sir Charles Lyell than of any other man at all approaching him in eminence, and he looked back on this friendship "with unalloyed satisfaction as one of the most instructive and enjoyable episodes" in his life. (4, I, 435.)

Speaking of Lyell's liberality and breadth of view, and his willingness to listen to an opponent, Wallace says:—"This was well shown in the time and trouble he gave to the discussion with myself as to the glacial origin of the larger alpine lake basins, writing me one letter of thirty pages [March, 1869, also another of thirteen pages in the same month] on the subject. Considering his position as the greatest living authority on physical geology, it certainly showed remarkable open-mindedness that he should condescend to discuss the subject with such a mere amateur and tyro as I then was." (4, I, 433.)

Wallace's innate diffidence and modesty is conspicuous in his account of another friendship. "Although Huxley was as kind and genial a friend and companion as Darwin himself, . . . yet I never got over a feeling of awe and inferiority when discussing any problem in evolution or allied subjects--an inferiority which I did not feel either with Darwin or Sir Charles Lyell. This was due, I think, to the fact that the enormous amount of Huxley's knowledge was of a kind [anatomy and physiology] of which I possessed only an irreducible minimum, and of which I often felt the want. . . . And because I was thus ignorant, and because I had a positive distaste for all forms of anatomical and physiological experiment, I perhaps over-estimated this branch of knowledge and looked up to those who possessed it in a pre-eminent degree as altogether above myself." (4, II, 39.)

The feeling of which Wallace spoke was, as I have said, the product of his natural and pronounced diffidence. In the life of the tropics, with all its luxuriance and infinitely complex and varied relationships, Wallace was as much a master as Huxley in his own special researches--Huxley as little an authority and as little interested as was Wallace in *Comparative Anatomy*; yet we may be sure that Huxley's feelings, if he had thought about the matter at all, would have been one not of inferiority but of regret,—the regret he expressed to Hooker, who was admiring the lovely grasshoppers in

\* In "The Lower Slopes," John Lane, London.

the Rhone Valley: "I would give anything to be as interested in them as you are."\*

As one more example of Wallace's appreciation of the work of others I may quote from a letter written to me, June 13, 1897, from Parkstone, expressing his admiration of "Prichard's wonderful anticipation of Galton and Weismann!† It is so perfect and complete. . . . It is most remarkable that such a complete statement of the theory and such a thorough appreciation of its effects and bearing should have been so long overlooked. I read Prichard when I was very young, and have never seen the book since. His facts and arguments are really useful now, and I should think Weismann must be delighted to have such a supporter come from the grave. His view as to supposed transmission of disease is quite that of Archdall Reid's recent book. He was equally clear as to Selection, and had he been a *zoologist* and *traveller* he might have anticipated the work of both Darwin and Weismann!" (6, II, 73.)

Wallace's appreciative sympathy was not only called forth by men of great eminence and learning, it also went out to those who were young and unknown.

"A keen young naturalist in the north of England, taking part in an excursion to the New Forest, had called on Wallace and confided to him the dream of his life—a first-hand knowledge of tropical nature. When I visited 'Old Orchard' in the summer of 1903, I found that Wallace was intently interested in two things: his garden, and the means by which his young friend's dream might best be realised. . . . The subject was referred to in seventeen letters to the present writer; it formed the sole topic of some of them. It was a grand and inspiring thing to see this great man identifying himself heart and soul with the interests of one—till then a stranger—in whom he recognised the passionate longings of his own youth. By the force of sympathy he re-lived in the life of another the splendid years of early manhood." (1, 348; 6, II, 227-8.)

In Wallace's correspondence with Darwin (6), of which it is only possible to refer to a small part, it is interesting to find the modern and inexact statement that Darwinism depends upon "infinitesimal" variations anticipated by Mivart and replied to by Chauncey Wright in 1871. A letter from Wallace to Darwin, written July 12, 1871, points out that "Mivart's greatest error, the confounding 'individual variations' with 'minute or imperceptible variations,' is well exposed by C. Wright. . . . Mivart . . . has told me that he was sorry the word 'infinitesimal,' as applied to variations used by Natural Selection, got into his book, and that he would alter it. . . ." In the same letter Wallace says that he always thought Darwin "laid too much stress on

\* "Life and Letters of T. H. Huxley," by Leonard Huxley, London, vol. II, p. 443 (1900).

† Described "Science Progress," vol. VI, new series, vol. I, p. 278 (1897).



the slowness of the action of Natural Selection owing to the smallness and rarity of favourable variations." (6, 1, 267.)

The chief differences between Darwin and his friend, as set forth in Wallace's "Life" (4, 11, 16-22), are so interesting and important that a detailed treatment is appropriate. They are arranged under four heads, of which the first, concerned with the nature and evolution of the human mind, is here considered last because it leads naturally to Wallace's convictions on spiritualism and other subjects. The three remaining heads follow Wallace's arrangement.

*Sexual Selection through Female Choice.*—That part of Darwin's theory which includes the combats between the males was held by Wallace "as strongly and as thoroughly" as by Darwin himself, but he looked upon the development of weapons—horns, canine teeth, spurs, etc.—as a result not of Sexual but of Natural Selection acting through such combats. The second part of Sexual Selection—the development of male ornament or song by the exercise of female choice he at first accepted, then doubted and finally rejected altogether. In 1869, before he published anything on the subject, Wallace explained his position in a very interesting correspondence with Darwin. In this he argued that the difference between the colouring of male and female was to be explained by the greater needs, greater danger, and greater value of the female, and the fact that variations appearing in one sex may be transmitted to that sex only. These two principles together enabled Natural Selection, he argued, to act on the two sexes as if they were two species. He also pointed out that in the specially protected groups the warning colours of the sexes were usually alike.

To this general argument he added a special one, viz., that, in the weak-flying Pierine butterflies of the genus *Leptalis*, both sexes mimic the "*Heliconidæ*" (*Ithomiinae*), while in the stronger-flying *Papilio*, *Pieris* and *Diadema* the female only is the mimicker. With the extension of knowledge this argument has collapsed. The males of the Ethiopian *Diademas* (*Hypolimnas*), often called *Euralias*, are just as good mimics as their females, and the same is true of nearly all mimicking species in the large section of swift-flying *Papilios* known as "Kite-Swallowtails" (*Cosmodesmus*), to which the well-known European "Scarce Swallowtail" (*P. podalirius*) belongs.

The fact that Wallace's statement holds good in one section but not in another of *Diademas*, and in one but not in another of *Papilios*, proves that the absence of mimicry in the males is not related, as this great naturalist argued, to strength of flight, but to the sex-limited inheritance of the female pattern. For in the other sections with equal, or in *Papilios* probably superior, powers of flight, these patterns are not sex-limited and the males are mimics no less than the females. Wallace's "special argument" cannot therefore be sustained, although the "general argument" holds good and probably explains, at least in part, why the bright colours and other secondary sexual characters of males

remain sex-limited, and, also in part, why female butterflies gain mimetic patterns so much oftener than males.

Wallace's alternative explanation of male song and male beauty of form or colour as the expression of superabundant energy and vitality breaks down, in the opinion of the present writer, directly an attempt is made to determine the physical causes of these characters. There are no grounds for the belief that a pigment which absorbs all the rays of the spectrum except red or blue is an indication of energy and vitality any more than one which absorbs them all and appears black.

Another special difficulty felt by Wallace was the presence, on the wings of male butterflies, of plumules or battledore scales "almost invariably changing in form with the species and genera in proportion to other changes, and always constant in each species yet confined to the males, and so small and mixed up with the other scales as to produce no effect on the colour or marking of the wings. How could Sexual Selection produce them." (6, I, 244-5.)

To this Darwin could make no answer but only include them in the number of unexplained sexual characters. (6, I, 245-6.) But the observations and histological researches of many naturalists in recent years—Dixey, Longstaff, Lamborn, Geoffrey Carpenter, Eltringham and Martin Mosely—founded on the classical work of Fritz Müller, have shown that these peculiar scales are scent-producing organs and have demonstrated the structure and use of the elaborate mechanism which, in many species and not in butterflies only, collects and distributes their secretion. They supply, in fact, what is probably the strongest of all evidence in support of the Darwinian theory of Sexual Selection.

*Arctic Plants in the Southern Hemisphere, and on Isolated Mountain-tops within the Tropics.*—Wallace states that he "was obliged to reject Mr. Darwin's explanation of the above phenomena by a cooling of the tropical lowlands of the whole earth during the glacial period to such an extent as to allow large numbers of north-temperate and Arctic plants to spread across the continents to the southern hemisphere, and, as the cold passed away, to ascend the summits of isolated tropical mountains." (4, II, 20.) Darwin held this belief "that the whole world was cooler during the glacial period," at least as far back as 1866, when he used these words in a letter to Lyell and urged as evidence the "many temperate plants on the summit of Fernando Po, and on the mountains of Abyssinia. I look on it as certain" he continued, "that these plants crossed the whole of Africa, from east to west, during the same period." (4, II, 21.)

Wallace, on the other hand, having come to the conclusion that the flora of oceanic islands was chiefly due to the transmission of seeds by birds or by gales and storms, extended this explanation to the migration of plants along mountain ranges and from mountain-top to distant mountain-top. These

conclusions were really a development from Darwin's own teaching; for, in the Bornean paper already referred to (p. xiv), Wallace had contemplated continental extension to oceanic islands as the means by which they had received their fauna and flora. Against this explanation Darwin was prepared to "do battle to the death," and, concerning it, to "withstand the almost preternatural sagacity of Lyell." (3, II, 109-10.)

Both Bates and Wallace felt that the cooled tropical belt, required by Darwin's hypothesis, "would inevitably have destroyed much of the overwhelming luxuriance and variety of plant, insect, and bird life that characterize those regions." (4, II, 20.) Wallace also argued that the differences between the southern and northern forms proved the existence of what Sir Joseph Hooker has called "a continuous current of vegetation from north to south"—a current which began to flow long before the glacial period.

Another argument which has strongly impressed the present writer is founded on the large northern element in the butterflies of southern South America, connected with the north by continuous mountain ranges, bridging over the tropics. No such means of distribution exists in the Old World, where the ranges are far less continuous and on the whole run east and west instead of north and south. In correspondence with these facts the northern element is far less evident at the southern extremities of the Old World, Tasmania and the Cape, than it is at the extremity of the New.

New Zealand is especially interesting in relation to this discussion; for its *Lepidoptera*, both butterflies and moths, bear a marked northern facies, best explained by transmission from the south of South America (and indirectly from the north) by means of an extended and warmer Antarctic continent and islands.

This divergence between the views of Darwin and Wallace—a divergence which in the opinion of Sir William Thiselton-Dyer "does not lie very deep"—is without any bearing upon the theory of Natural Selection. It was, nevertheless, keenly felt by Darwin, who wrote in a letter of January 2, 1881, about it and other points of divergence in their views:—"How lamentable it is that two men should take such widely different views, with the same facts before them; but this seems to be almost regularly our case, and much do I regret it." (4, II, 13-4.) After quoting this letter Wallace remarked—"It is really quite pathetic how much he felt difference of opinion from his friends. I, of course, should have liked to have been able to convert him to my views, but I did not feel it so much as he seemed to do. (*Loc. cit.*)

*Pangenesis, and the Heredity of Acquired Characters.*—It is hardly reasonable to speak of Wallace's views on this subject as a difference between Darwin and him. During Darwin's lifetime the effects of use and disuse and other "acquired characters" were believed to be hereditary by naturalists in general,

\* "Darwin and Modern Science," edited by A. C. Seward, Cambridge, p. 305 (1909).

including Wallace himself. Darwin believed in such transmission and he suggested Pangenesis mainly in order to explain it, as is clearly shown in many of his writings and especially in the following sentence from a letter to Huxley about 1865:—"I think some such view will have to be adopted, when I call to mind such facts as the inherited effects of use and disuse, etc." (3, III, 44.)

Darwin did not admit that Galton's transfusion experiments with rabbits disproved the existence of gemmules,\* but Weismann's challenge to the belief in the transmission of acquired characters and his theory of the Continuity of the Germ-plasm came later, and it can hardly be set down as a difference with his illustrious comrade that Wallace accepted conclusions and a hypothesis of which Darwin had never heard. And, as we know from the following letter to Lyell (February 20, 1868), Wallace himself in earlier years warmly accepted Pangenesis and maintained that it could never be disproved: "The hypothesis is *sublime* in its simplicity and the wonderful manner in which it explains the most mysterious of the phenomena of life. To me it is *satisfying* in the extreme. I feel I can never give it up, unless it be *positively* disproved, which is impossible, or replaced by one which better explains the facts, which is highly improbable. Darwin has here decidedly gone ahead of Spencer in generalization. I consider it the most wonderful thing he has given us, but it will not be generally appreciated." (4, I, 422.)

*The Origin of Man as an Intellectual and Moral Being.*—Darwin's conclusions are thus stated by Wallace:—"Man's whole nature—physical, mental, intellectual, and moral—was developed from the lower animals by means of the same laws of variation and survival; and, as a consequence of this belief, . . . there was no difference in *kind* between man's nature and animal nature, but only one of degree." Wallace believed on the contrary, "that there is a difference in kind, intellectually and morally, between man and other animals; and that while his body was undoubtedly developed by the continuous modification of some ancestral animal form, some different agency, analogous to that which first produced organic life, and then originated *consciousness*, came into play in order to develop the higher intellectual and spiritual nature of man." Of these views Wallace truly says—"they do not in the least affect the general doctrine of Natural Selection. It might be as well urged that because man has produced the pouter-pigeon, the bull-dog, and the dray-horse, none of which could have been produced by Natural Selection alone, therefore the agency of Natural Selection is weakened or disproved. Neither," he urged, "is it weakened or disproved if my theory of the origin of man is the true one." (4, II, 17.)

This theory was first made known in 1864 and was hinted at by Darwin in

\* "Nature," vol. III, p. 502 (April 27, 1871); see also Galton's reply in vol. IV, p. 5 (May 4, 1871).

a letter of November 22, 1870, referring to his "Descent of Man"—"I . . . am half-way through proofs of 2nd vol. of my confounded book, which half kills me by fatigue, and which I fear will quite kill me in your good estimation." (4, II, 7.) On this Wallace comments:—"I never had the slightest feeling of the kind he supposed, looking upon the difference as one which did not at all affect our general agreement, and also as being one in which no one could dogmatize, there being much to be said on both sides."

The last letter received by Wallace from Darwin was written July 12, 1881, less than a year before his death on April 19, 1882. Wallace had directed his attention to Henry George's "Progress and Poverty"—on which Darwin, after saying that he would certainly order it, made the amusing comment—"I read many years ago some books on political economy, and they produced a disastrous effect on my mind, viz., utterly to distrust my own judgment on the subject, and to doubt much every one else's judgment!" (4, II, 14.)

Wallace, quoting the kindly discursive letter in full, says of the friendly feeling shown in it—"to have thus inspired and retained it, notwithstanding our many differences of opinion, I feel to be one of the greatest honours of my life."

Hence of the four differences recounted by Wallace and looked upon by him as the only important ones, it is only of the first (as arranged here) that we are able to say unreservedly that he was "more Darwinian than Darwin himself" (4, II, 22); the second has no bearing on the general theory of Natural Selection; the third followed from ideas which were necessarily unknown to Darwin; the fourth from Wallace's belief that Natural Selection was inoperative where Darwin believed it to be efficient, but in a special instance which left the general theory untouched.

An interesting letter to the present writer states conclusively the grounds upon which the last difference with Darwin was built. Wallace had asked me to read "and criticise" part of his concluding chapter on Man, in "Darwinism." After receiving some notes he wrote February 22, 1889:—

"Many thanks for your kindness in looking over my proofs. I will not trouble you with the last sheet, which would only horrify you still more. I am quite aware my views as to Man will be—as they have been—criticized. I have referred to Weismann's opinion further on; but I doubt if his view or yours will really account for the facts. Of course we look at the question from different standpoints. I (think I) *know* that non-human intelligences exist—that there are *minds* disconnected from a physical brain—that there *is*, therefore, a *spiritual world*. This is not, for me, a *belief* merely, but *knowledge* founded on the long-continued observation of facts—and such *knowledge* must modify my views as to the origin and nature of human faculty." (2, 470-1.)

We are thus led to the grounds of his convictions on spiritualism and to consider whether his mind was of the type best suited to test the proofs that are offered, and to guard against trickery and fraud.

Wallace had, as his friend and neighbour at Godalming remembers, "apparently unfailing confidence in the goodness of human nature. No man nor woman but he took to be in the main honest and truthful, and no amount of disappointment—not even losses of money and property incurred through this faith in others' virtues—had the effect of altering this mental habit of his." (6, II, 109.)

He wrote of de Rougemont, "I firmly believe that his story is substantially true." (6, II, 76.) He wrote to me, June 3, 1913, a few months before his death, expressing the belief that the author of a pamphlet, now known to be an ingenious forgery,\* "was too earnest, and too clear a thinker, to descend to any such trick." (2, 470; 6, II, 100.) That he could be very credulous and uncritical when his sentiments were concerned is impressively shown by his statement that "thousands of dogs' lives are sacrificed annually to establish some minute point in physiology" (4, II, 392), an inaccuracy to which Lord Avebury drew his attention. (6, II, 212.)

Among the many descriptions of séances and the behaviour of mediums given by Wallace there is an account of two meetings, in 1886 or 1887, in the house of a spiritualist in Boston at one of which the late Prof. William James was present. (4, II, 337-41.) The phenomena described by Wallace seem to a disinterested reader suspicious in the highest degree, but he eagerly accepted everything at its face value. This is clear from his description, but it was also evident at the time to the critical mind of William James. I happened to meet the late Prof. Josiah Royce at Baltimore in 1909 and he told me that, as he and James were walking back to Harvard, after the séance, his friend remarked:—

"It is a curious thing to see Wallace plunging head foremost into a flood which we Americans only allow just to wet our feet."†

In some cognate subjects Wallace displayed a critical insight. He rejected theosophy and the hypothesis of a "subconscious self." (6, II, 205, 213.) "I am quite astonished," he wrote to a friend, "at your wasting your money on an advertising astrologer. In the horoscope sent you there is not a single definite fact that would apply to you any more than to thousands of other men. All is vague, what 'might be,' etc. etc. It is just calculated to lead you on to send more money, and get in reply more words and nothing else." (6, II, 215.)

Speaking of "the most regrettable incident" in his life—the wager with John Hampden which, although he won it, cost him "fifteen years of continued

\* "Proc. Linn. Soc. Lond.," p. 26 (1912-13); p. 23 (1913-14).

† To confirm an unaided memory I wrote to Prof. Royce some years later and received a reply which confirmed the substance of the remark as quoted above.

worry, litigation, and persecution, with the final loss of several hundred pounds," he says—"it was all brought upon me by my own ignorance and my own fault—ignorance of the fact so well shown by the late Professor de Morgan—that 'paradoxers,' as he termed them, can never be convinced, and my fault in wishing to get money by any kind of wager." (4, II, 364.) But de Morgan's conclusion, so well applied by Wallace to the flat earth champion, also forces itself upon us when we think of the great man who considered that his arguments for anti-vaccination were "about the most demonstrative bit of work" he had done. (6, II, 206.)

Wallace was doubtless referring to this and the other "anti" causes so dear to him when he confessed to Mr. J. W. Sharpe that his intellectual interests "were agreeably stimulated by novelty and opposition." His friend remembered too that "an uphill fight in an unpopular cause, for preference a thoroughly unpopular one, or any argument in favour of a generally despised thesis, had charms for him that he could not resist." (6, II, 109.)

Wallace lived in many places after his return from the East in the spring of 1862; at first with his brother-in-law in Westbourne Grove, and then in 1865 with his mother in St. Mark's Crescent, Regent's Park. After his marriage in 1866 he lived in London for four years, but let his house for one of these which he spent at Hurstpierpoint, with his wife's family.

In 1870 he took a small house at Barking, and in the following year leased four acres of land, including an old chalkpit, at Grays, on the Thames, twenty miles from London. Here he built "The Dell," using as far as possible the materials on the ground and superintending much of the work himself. He was unfortunately cheated by a dishonest builder who had contracted for the building but failed to complete it, left Wallace to pay for materials he had obtained on credit, and finally brought an action for damages because he was not allowed to finish the work he had abandoned. In the action which ensued Wallace was successful and was given costs on every point, but nothing was ever recovered and he was left "to pay about £100 law costs for what was merely an attempt to extort money." (4, II, 364.) He occupied the house in March, 1872, and lived in it for four years during which he wrote "The Geographical Distribution of Animals," urged, he believed, to undertake the task by his friends, Professor Alfred Newton and Dr. Philip Sclater, whose Zoological Regions he followed. Wallace himself seemed to be somewhat disappointed with the work, thinking that "it was written a quarter of a century too soon" (4, II, 97), but I have always found it of the greatest value ever since the time when, a few months after its appearance in 1876, I chose it as part of a college prize. At Grays, too, began the keen interest in his garden, which was such a pleasure to him up to the end of his life.

The memories of his son and daughter (6, II, 103-138), beginning with life at "The Dell," tell of Wallace's love for his home wherever it was, and how he

took the keenest interest in all arrangements, even the details of cooking, and liked to do as much as possible with his own hands—a result due in part to his travels, but also inherent. He was not particular about his personal appearance, in clothes thinking chiefly of comfort. He delighted in walks with his family and explaining to them the interest of the plants and animals they met. One among many interesting and often amusing memories (p. 126) must not be omitted, although it belongs to a much later time when he lived at Broadstone. Two young pupils of his daughter, great friends of his, disputed about the number of the cow's stomachs and wrote to him for a decision.

"Dear Irene and Reggie" he replied, "your dispute . . . can be settled and rectified by a simple mathematical process usually called subtraction, thus:—

Irene's Cow .....	7 stomachs
Reggie's Cow .....	3 stomachs
 The Farmer's Cow .....	 4 stomachs."

The house at Grays was sold in 1876 and he first moved to Dorking for two years and then to Croydon, where he wrote "Island Life," that fine continuation of "Geographical Distribution."

In 1881 Wallace moved to "Nutwood Cottage," which he had built at Godalming. At this time his financial difficulties were at their worst and a Civil List Pension of £200 came as a great relief. His friend, Miss Arabella Buckley (Mrs. Fisher), informed Darwin of the circumstances and he, with the co-operation of Huxley and other scientific friends, approached the Prime Minister (4, II, 378). Darwin wrote to Miss Buckley on the subject:—"I hardly ever wished for anything more than I do for the success of our plan." (3, III, 228.)

I first met Wallace at Godalming, being taken to call when on a walking tour with a dear mutual friend, Raphael Meldola, who had long known him. At "Nutwood Cottage" Wallace had much friendly intercourse with the Charterhouse masters. One of them, his friend and neighbour, Mr. J. W. Sharpe, remembers that "nothing made him happier than some plan for reforming the house, the garden, the kitchen-boiler, or the universe. . . . Successful or not, he was always confident that the next would turn out to be all that he expected of it." (6, II, 108.)

It was at Godalming that he wrote "Darwinism," the book having been suggested to him by the success of a lecture with this title delivered on his American tour in 1886-7. The Hon. D.C.L. Degree of Oxford University was offered to Wallace for the Encaenia of June, 1889, just as he was arranging to leave Godalming. His disinclination for public ceremonials led him to write on May 28:—



"I have just received from Prof. B. Price the totally unexpected offer of the Hon. Degree of D.C.L. at the coming Commemoration, and you will probably be surprised and *disgusted* to hear that I have declined it. . . . The fact is, I have at all times a profound distaste for *all* public ceremonials, and at this particular time that distaste is stronger than ever." Then after speaking of the pressure on his time due to the imminent move from Godalming to Parkstone and other engagements, he continued, "Under these circumstances it would be almost impossible for me to rush away to Oxford except under absolute compulsion; and to do so would be to render a ceremony which at any time would be a trial—a positive punishment.

"Really the greatest kindness my friends can do me is to leave me in peaceful obscurity, for I have lived so secluded a life that I am more and more disinclined to crowds of any kind." (6, II, 217-8.)

When it was proposed that the degree should be conferred in the autumn instead of at the Encaenia he felt that he could not refuse, but hoped that it would not be urged\* upon the Council of the University "as I really feel myself too much of an amateur in Natural History, and altogether too ignorant (I left school—a bad one—finally, at 14) to receive honours from a great University. But I will say no more about that." (*Loc. cit.*)

A few years later he refused, for the same reason, to unveil a statue of Darwin in the Oxford University Museum, and I well remember the sly humour with which he hinted that Sir Joseph Hooker would be a far more appropriate central figure at the ceremony. (2, 469.) And when he was asked to take part in the Oxford celebration of the Centenary of Darwin's birth he replied:—"All the *attractions* of your celebration, are, to me *repulsions*. If I ever do come to Oxford it will be for a very short visit to *you* alone, and to see your great series of illustrations of mimicry."

He had indeed taken a splendid part on July 1, 1908, in the Linnean Society's celebration of the 50th anniversary of the Darwin-Wallace Essay, but I believe that he was moved by a sense of duty to disregard his own preferences. His words on that historic occasion showed that he felt it to be a unique opportunity of paying homage to the mighty genius whose name had been, and will ever be, associated with his own. (2, 468-9.) No spectator of that inspiring scene can ever forget it, or the words in which Wallace spoke of Darwin and himself—of how the idea of Natural Selection came to both in a sudden flash of insight, by him written down, copied and sent off within one week. Then, contrasting his own actions with Darwin's twenty years of research, he went on:—

"I was then (as often since) the 'young man in a hurry': *he*, the painstaking and patient student, seeking ever the full demonstration of the truth that he had discovered, rather than to achieve immediate personal fame. . . .

\* Wallace was here mistaken in his ideas about the procedure. The Council had already decided to propose the Hon. Degree and the vote was valid for any convenient occasion as well as for the Encaenia.

If the persuasion of his friends had prevailed with him, and he had published his theory, after ten years'—fifteen years'—or even eighteen years' elaboration of it—I should have had no part in it whatever, and *he* would have been at once recognised, and should be ever recognised, as the sole and undisputed discoverer and patient investigator of the great law of 'Natural Selection' in all its far-reaching consequences."\*

It was doubtless the same love of simplicity, so largely bound up with his dislike of ceremonial, which led Wallace to oppose the use of new technical terms or of a new terminology. The present writer well remembers his objection to the words derived from the Greek, suggested in 1890 in order to bring precision into the study of animal colours and their meaning. Before their introduction the concealing shapes, colours and patterns were generally spoken of as examples of "Protective Resemblance," and yet an animal with conspicuous Warning Colours, belonging to the opposite category, was said to belong to a "Protected Group." Again, the ordinary use of the word "Mimicry," implying conscious imitation, led to continual misunderstanding when the same term was employed in science for superficial resemblance developed by Natural Selection, while further confusion arose from its technical use by some naturalists following Bates, to include, by others following Wallace, to exclude Protective Resemblance. Such difficulty and confusion, inevitable when ordinary words are employed in a technical and limited sense, are at once overcome by the use of new terms, chosen for the purpose. Nevertheless, their introduction was certainly distasteful and seemed to be even painful to Wallace.

After several years at Godalming Wallace began to long for a milder climate and a garden more open to the sun. He fixed upon Parkstone and took a house there in 1889, although he could not be long content without planning alterations. A charming and amusing letter to his son shows the great interest he took in carrying out all the fascinating details. (6, II, 111.) I remember admiring the slopes in his garden and regretting the difficulty of getting such interesting effects in level ground. "Oh no," he said with his usual cheery optimism, "it is perfectly easy and inexpensive. Every foot you excavate means a foot added at the top, so there's a double result for each bit of work."

It was during his residence at Parkstone that Wallace was present for the last time and read his last paper at an ordinary meeting of a scientific society. It was a contribution to the discussion on "The Utility of Specific Characters" at the Linnean, June 18, 1896. I remember with great pleasure a breakfast next day at Professor Meldola's. The party, which also included Sir Francis Darwin, sat and talked until far into the morning. Finally as Wallace rose he said "Well, I should like to go on in this way all day!" (2, 470.) I remember

\* Darwin-Wallace Celebration of the Linnean Society of London, p. 7 (1908).

too his dislike of some scientific heresy—he was as a rule very tolerant of heresies!—and the vigour with which he said—“I cannot away with it!”

Wallace was elected F.R.S. in 1893, but his friends had some difficulty in obtaining his consent to the nomination, and interesting letters on the subject passed between him and Sir William Thiselton-Dyer. (6, II, 219-222.) He received the Copley, the Royal and the Darwin medals of the Society, the last awarded on its inauguration. He also received the Darwin-Wallace Gold Medal of the Linnean Society of London, at the 50th anniversary of the joint essay, on July 1, 1908. The Order of Merit was conferred on him in the same year.

The growth of buildings close at hand made Parkstone unsatisfactory and after living ten years there Wallace began to look for another and less crowded spot. After much searching he finally bought in 1901 a site after his own heart at Broadstone, only four miles from Parkstone. Here he built “Old Orchard,” employing the men and buying the materials himself. In May, 1903, he wrote of “the charming ‘lodge in a wilderness’ I have got here in which to end my days on earth. I assure you I am enjoying it, perhaps more than I should ever have done at an earlier period.” (1, 348.)

It was at Old Orchard that I came to realise the extraordinary keenness and vitality which kept him young up to the end of his long life, so that to his children he never grew old and to hear him described as “a wonderful old man” was quite a shock. (6, II, 111.) And his youth appealed to others who saw him for the first time. “I’ve enjoyed every minute of the time. Why, he has the spirit of a boy of eighteen!” So said one of my daughters, herself about that age, as we left the gate of Old Orchard. (1, 348.)

And he expected the same keenness and love of work in others. “Many happy returns and lots of work” was his birthday greeting in 1909. (*Loc. cit.*)

His most intense interest and the one which most of all kept him young was gardening. This was always “pure enjoyment.” He never made experiments on plants, never attempted to study their minute structure or to write about them; the mere seeing them grow, noting the infinite diversities of their forms and habits, their likes and dislikes, was to him a delight in itself. (4, II, 204.)

An extract from a letter of March 13, 1911, in his 89th year, brings this intense keenness before us. “What I am mainly at work (or at *play*) upon now is my garden, and I have suddenly developed a sad *mania* for Alpine plants, more especially for my old favourites the genus *Primula* which has received such wonderful additions lately from the Himalayas, but more particularly from *N. China*. My resuscitated hobby is due to my having now—the very *first* time in my life—a bit of ground really *suitable* for them, combining *shelter*, good aspects, a moist (even *boggy* in parts) sub-soil, a moister atmosphere, and a good and varied soil. . . . I have already got such a fine lot of plants—about 20 sp. of *Primulas* and 150 of *Alpines* generally—with promises

of more—that I am laying out a regular Alpine and bog garden, on a quite small scale, buying stone and stone chippings by the ton or truck-load, collecting sand and road scrapings, protecting against rabbits, etc., which all give me very interesting occupation, so filling up my time and powers of work that I have little time or energy for reading anything but newspapers, novels, and the regular supply of scientific or political periodicals” (in part from 1, 348). And later on in the same year of wonderful sunshine he wrote, August 23, lamenting the three months’ drought—“I was in the midst of making my new Alpine garden in the beginning of it! This I shall still be glad to show you as I have a lot of interesting things which I hope will be fine another year. . . . My *Tecoma radicans* is a sight, about 40 flower bunches on it, and I hope some will be on when you come. . . . If we do but get average weather next summer I hope to see a number of my best things in flower, which I have been waiting for this 5 to 10 years.”

This absorbing occupation did not lessen the interest in mankind which Wallace shared with Darwin. It will be remembered that the older naturalist, although, quoting Cobbett, he spoke of the “bloody old *Times*,” still said that he could not do without it and that it was “meat and drink” to him. Just a year before his death Wallace wrote, November 12, 1912:—“I am—for me—very busy now with two SMALL books on hand, . . . But I now have to work very slowly, and the *war-news* every day MUST be read.” (2, 469-70.)

Just after his 91st birthday he wrote in answer to the congratulations of his doctor:—“I am glad to say I feel still able to jog on a few years longer in this *very good* world—for those who can make the best of it.” (6, II, 136.)

After nearly a week of gradually increasing weakness he died painlessly on November 7, 1913.

Men will be held in honoured remembrance for their positive contributions to “the advancement of learning,” and, if some of Wallace’s thoughts fail to convince the scientific world, there remains enough of mighty achievement to make sure for him a high place in the temple of fame.

1. ‘Nature,’ vol. XCII, November 20, 1913, pp. 347-9. E.B.P.
2. ‘The Zoologist,’ Ser. 4, vol. XVII, December 1913, pp. 468-471. E.B.P.
3. ‘The Life and Letters of Charles Darwin, including an Autobiographical Chapter.’ Edited by Sir Francis Darwin, vols. I-III, 5th thousand, revised. London, 1887.
4. ‘My Life, a Record of Events and Opinions,’ vols. I, II, by Alfred Russel Wallace. London, 1905.
5. New and condensed edition of the above, in one volume. London, 1908.
6. ‘Alfred Russel Wallace, Letters and Reminiscences,’ vols. I, II, by Sir James Marchant. London, 1916. E.B.P.

## DAVID SHARP, 1840-1922.

DAVID SHARP was born at Towcester, August 15, 1840, but the early years of his life were passed at Stoney Stratford. When he was about eleven years old, his parents moved to North London, where, after attendance at preparatory schools, he entered St. John's Foundation School at Kilburn, in 1853. After he had left school, in 1857, a short experience of his father's business in London proved that a commercial life was not at all congenial. In 1862, he entered St. Bartholomew's Hospital as a medical student. One may suspect that this choice of a medical career was more or less of a compromise, for it is certain that his own inclination was to obtain a post in a museum, while his father wished him to engage in some business of a commercial character.

Very little is known of incidents in Sharp's early life which have any bearing on his later scientific work, but before he entered St. Bartholomew's he had already formed a considerable collection of beetles—that group of Insects which was to become the object of his special study throughout his long life. Originally, we are told, his interest had been drawn to Insects by the beauty of butterflies and moths, and that, with the help of one of his sisters, he used to breed these in a top room of his father's house in St. John's Wood. Here he came under the influence of Herbert Spencer, who for a time lived in the same house, and encouraged and helped him with his studies in Natural History. Spencer's influence, no doubt, made a strong and lasting impression on Sharp's mind, and many years afterwards on this philosopher's death he wrote an article "On the Place of Herbert Spencer in Biology."

Mr. F. Muir, in his Obituary Notice, tells us that an old note-book of Sharp's shows that, in 1863, he possessed 662 named species of British beetles, and that in two years his collection had increased to 1,984. The smaller number would indicate that, in the year mentioned, he had not collected Coleoptera very long, or, at least, not as a speciality; possibly Lepidoptera had not yet ceased to be desirable objects of pursuit. Already, however, he was publishing not only lists of his captures, but also instructions for mounting and preserving Coleoptera, so that he must have had considerable experience. That Sharp, with his keen appreciation of small structural differences, should have turned from the Lepidoptera, as classified in those days, to another group, in the study of which that appreciation was of the highest value and importance, was quite natural. Many years later he remarked that, were he making a collection of Lepidoptera, it would consist of specimens so denuded that all structures could be plainly seen.

At no time can Sharp have been more fully occupied than in those four years when he was studying medicine, first, as above mentioned, in London, and, later, for another two years at Edinburgh, where he took his degree M.B.

and C.M., in 1866. Just at this period there happened to be a very energetic band of Coleopterists in England, some of them hardly more than collectors, but others as efficient in the study as they were in the field; G. R. Crotch, Waterhouse, Rye, Matthews, Blackburn, Janson and Power, being amongst these. Sharp very soon showed himself to be not less capable than the best of them. One is led to wonder how all the entomological work he achieved can have left time for the necessary medical studies during his student days! The collecting, preparation, and working-out of his specimens, the description of species either altogether new, or new to the British Fauna, might well have occupied a man's whole time. Moreover, he collected far and wide in these days, for, in 1864, we read of the rarities, or novelties, that he found not only in localities near London (Wimbledon, Weybridge, etc.), but also at Rannoch, in Scotland, with his friend Bishop, in the Fen country at Horning, with Brewer, and on the East Coast at Deal.

After obtaining his degrees, for a short time Sharp assisted a friend in a London practice, but, in 1867, he returned to Scotland, having obtained an appointment as medical officer in the Crichton Institution at Dumfries. While thus employed he found time to produce a really important piece of work, the 'Revision of the British Species of *Homalota*,' published in 1869. His friend Rye speaks of it shortly before its publication as "a very difficult work which Dr. Sharp has now nearly completed . . . , for which purpose he has burdened himself with the difficulties, or with the entire collections, of a very great number of British and Continental entomologists"; of the same paper Commander Walker has recently written that "to this day, it remains the foundation of our knowledge of the extensive and difficult group of beetles included under the generic name."

From the Crichton Institution he moved to Thornhill, in charge of a wealthy patient, who remained in his care for years, dying in 1883. While thus engaged he was able to devote nearly his whole time to Entomology. He soon turned his attention to the study of foreign Coleoptera, publishing several papers of primary importance, the "Staphylinidæ of Japan," in 1874, and of the "Amazon Valley," in 1876, and his Monograph "On Aquatic Carnivorous Coleoptera, or Dytiscidæ," in 1880-82. In 1874, we find Rye deploring the decrease in additions to the British Coleoptera during the two or three preceding years, and attributing this to the fact that "some of our best men have not found enough to satisfy their abilities and energies in the fauna of our own country." This remark must have been due largely to Sharp's change to a wider field of study, for Rye credits him with no less than 38 additions to the British list in 1871, and with only about a dozen in the next three years together.

In 1876, T. Blackburn, who some ten years before had been Sharp's companion on various collecting expeditions in Scotland and had since become ordained, accepted a position under the Bishop of Honolulu in the Hawaiian Islands. During a residence of some years, encouraged by Sharp, he devoted all such time as he could secure from his official duties to the investigation of the

insect fauna and especially of the Coleoptera. The larger part of Blackburn's material of this Order of Insects was worked out by Sharp in a series of papers, followed in 1884 by an important publication under their joint authorship, entitled, "Memoirs of the Coleoptera of the Hawaiian Islands." The comments which follow the summary of the results are remarkable for Sharp's sound judgment as to the true nature of this beetle fauna, although the material on which he had had to base his conclusions was very deficient. Here too he strongly urges the investigation of this and other isolated faunæ as quickly as possible, both on account of their importance and of the danger of their destruction. At this time too he was deeply interested in the Fauna of New Zealand, and was laying down the foundation for a study of Coleoptera of that region.

On the death of the private patient who had been so long under his care, Sharp retired from further medical practice, and leaving Scotland resided for some time at Southampton. In 1888 he moved to Cambridge, and from 1890 to 1909 he was Curator of Insects in the University Museum of Zoology.

As Secretary of a Committee appointed for the purpose, he with the late Alfred Newton was mainly responsible for securing the means whereby the exploration of the fauna of the Hawaiian Islands, inaugurated by Blackburn and so strongly advocated by himself in 1884, might be continued. For more than twenty years he devoted an enormous amount of labour and thought to make this successful, himself working out and describing the material in several of the most important groups of Coleoptera and editing the 'Fauna Hawaiensis' in which these and other results were published. During these years at Cambridge he also undertook various large and important Families of Coleoptera for the 'Biologia Centrali-Americana,' describing therein the Water-beetles, Staphylinidæ, Brenthidæ, Bruchidæ, a large part of the Clavicorns and some of the Weevils.

So far we have referred only to some of Sharp's chief systematic and descriptive works dealing with Coleoptera, but in 1895 appeared the first volume of "Insects" in the Cambridge Natural History series, followed by the second four years later. Sharp's simple and lucid style was admirably adapted to the writing of such a work; his all-round knowledge of insects and of the literature of his subject such as no one in this country could approach. Occasionally (as in his account of the Grass-moths) that humour with which his friends are all so familiar peeps out. Westwood's "Introduction," published more than fifty years before and a marvellous work for its time, contained almost everything then known about insects, that its author could utilise. Sharp, with no greater space at his command, was embarrassed by the wealth of material from which he must select. At one time he expressed a hope that he might be able later to expand this work into one of many volumes, but the increased demands on his time made by the publication next noticed prohibited its fulfilment. As it is, the Orders of Insects first dealt with received so comparatively liberal an amount of space that the great group of Coleoptera, of which the author's knowledge was so

pre-eminent, occupies less than half the number of pages devoted to the Hymenoptera. No doubt this was partly due to the special interest of the habits of the latter and to the lengthy accounts of those taken from the works of Fabre, his favourite entomological writer—one might perhaps say his favourite author, since of all Fabre's works the charming little book written for little girls pleased him most. Sharp's "Insects" is justly popular, being invaluable to all Entomologists, and remarkable for the judgment used in the selection of its matter.

All Zoologists owe much to Sharp for his labour of thirty-seven years on the 'Zoological Record,' of which from 1885 he was Editor of the part "Insecta," and from 1891 of the whole. It is well known to all that he inaugurated great improvements in the 'Record.' There are those of his friends who would gladly have seen him give this up many years ago, partly because the freedom might have resulted in his writing a greatly enlarged, general work on Insects, and partly because combined with his other work the labour was often excessive and tried him severely. His letters too often confirm this: "The Damoclean 'Record' always in suspension," "My treadmill work at the 'Record,'" "The 'Record' becomes worse and worse," "I am worked to death with the 'Record,'" are significant remarks from so strenuous a worker.

In 1909 Sharp resigned his Curatorship and retired to the home he had built in the New Forest, at Brockenhurst. Here he passed the rest of his life, enjoying a great deal of outdoor work in this beautiful locality, and still making the most delicate and difficult studies of beetles, as keenly and accurately as in his youth. That most important paper "On the Comparative Anatomy of the Male Genital Tube in Coleoptera," by Sharp and Muir was published in 1912. In this work a division of the Coleoptera into eight great series or superfamilies was suggested and the Phylogeny of all the Families exhibited. As a result of their arduous investigations, which necessitated very numerous and often difficult dissections, the authors concluded that the structures described were of far greater value in Taxonomy and Phylogeny than Coleopterists had hitherto supposed. This conclusion as to the importance of such structures agrees with that expressed many years before by Edward Saunders concerning the Hymenoptera after a less elaborate examination of similar parts in insects of that Order. Nor did Sharp's interest in the subject cease after the publication of this paper, for six years later he writes: "I have been engaged for two years in the investigation of the sexual characters of the Rhynchophora . . . the results are so promising that I am preparing a big work on the subject. I expect Muir will be over here shortly, and will help, and, as my daughter is extremely skilful now, I hope we shall manage it, notwithstanding my years, which are now very serious."

Though most of his publications relate to Coleoptera, Sharp wrote occasionally on other insects, for instance, on the stridulation of ants and the structures by which these are produced; very rarely, as it appears, on non-entomological subjects, *e.g.*, the paper entitled "A Scheme for a National



System of Rest-Funds (or Pensions) for Working People" in 1892. In reality there were no branches of Entomology with which he was not conversant, for concerning those in which he was not himself engaged, he was fully informed through the wide extent of his reading, which was still further enlarged by his work on the 'Record.' Thus in 1903 he writes: "I have given much attention to Economic Entomology during the last twenty years, and of late specially. I am convinced that the internal parasites are the things that keep the balance of nature in the insect world fairly true. The predators are of value, but it is the internal parasites that are the great agents. They *are not seen*, and consequently we totally undervalue them, but I believe all insects may be kept down by the aid of well selected parasites." On theoretical and controversial questions that interest the biologist, his publications are mostly silent, though in "Insects," when treating of butterflies, he does devote some space to an unfavourable criticism of "mimicry." His cautious mind seemed never to admit the all-sufficiency of any of these theories, however widely accepted, presumably because, from the ample stores of his knowledge, he was always able to produce some fact or another which he considered irreconcilable with them.

Some of his shorter and less generally known entomological papers are of special interest, not only on account of the subject matter itself, but because consideration is given to theoretical questions that arise. Such is the paper on "The Modification and Attitude of *Idolum diabolicum*, a Mantis of the kind called 'Floral Simulators.'" This remarkable creature, which, by the resemblance of certain of its structures to a flower, attracts to itself other insects, destined to become its prey, is compared with other kinds of Mantis similarly attractive (but with the flower-like appearance due to different structures), and also with simpler forms. Comparison is also made of the attitudes and movements of the different species, with the result that these are considered to be of more importance than the petaloid structures, and to have been evolved before them. Although most naturalists would, at the time this paper was written, have, no doubt, considered these structures to have been developed by the action of Natural Selection, Sharp suggests that they "will ultimately be found to be due to the reactions between simple physical causes and the physiological processes of the creature," and he quotes from Herbert Spencer that "function is from beginning to end the determining cause of structure."

From the titles already cited, it will be seen that Sharp's writings are mainly descriptive, and his quick perception of even the most subtle differences between species made it easy for him to write descriptions of unusual efficiency. His acumen reminds one of that exhibited by C. G. Thomson when at his best in his descriptions of Scandinavian insects.

The extent of Sharp's work in the field gave him a great knowledge of insects as living creatures, and his success in this was due to his quick recognition of species, his cleverness in detecting special habits and habitats, to his retentive memory and great energy. When out collecting he was, as

Commander Walker writes, "a delightful companion, with an inexhaustible fund of dry humour under all circumstances." Most of his field-work was done in England and Scotland, but he also visited the Continent, and on one trip collected so successfully in Spain that, years after his return, he still had much material unmounted. For many years he cherished the hope of being able to visit Australia and examine the living creatures of that Fauna.

In collecting he by no means confined his attention to beetles, but, at times, paid very particular attention to other insects, and at one period was especially successful in his pursuit of Diptera. In 1888, and for some years after, he collected Hymenoptera in numbers, and many choice species amongst these, the most remarkable of his captures being the wasp, *Homonotus sanguinolentus*, a very striking addition to the British Fauna.

To young entomologists, and those of less experience than himself, Sharp was most liberal in giving his assistance and encouragement. Those about to visit other countries, whether as members of some expedition or independently, received from or through him the best advice not only as to the most profitable method of work, but also as to suitable equipment. In his room in the Cambridge Museum, he seemed always ready to turn from his own work and examine and give his opinion on any insect submitted to him, and to this room not only entomologists, but many zoologists came often in search of information. On account of his extraordinary knowledge of zoological literature and his familiarity with the resources of the Cambridge libraries, his help was in constant request. All these interruptions he took with great good nature, and some of us, we fear, at times took advantage of this and allowed him to hunt up information that we might, with a little more trouble, have obtained for ourselves! Though not writing specially on the subject of Economic Entomology, he was frequently receiving requests to recommend entomologists for economic work in distant parts of the Empire or in foreign countries, and put himself to much trouble to secure suitable men at a time when these were very difficult to obtain.

Amongst the friends of his earlier days were Spencer, Wallace, Huxley, Bates and Wollaston; later the American entomologists Riley and Horn visited him in England. He was much taken with the personality of the former, whose 'Missouri Reports' he valued highly, and subsequently urged him to apply for an entomological position of importance in this country. In reality Riley was by that time an almost spent force, so that one cannot regret that his hesitation removed any chance of success he may have

To entomologists it must be counted a real loss that Sharp has left no reminiscences of his early friends and colleagues, nor of his old collecting grounds, many of which are now much changed or have altogether disappeared as such. His accurate memory would have rendered these not only interesting but valuable. How retentive this was may be judged from the following: In 1865 (or perhaps previously), when collecting beetles, he captured, no doubt incidentally, a scarce ant (*Ponera coarctata*) in Surrey

More than 50 years after the event, when this capture was casually alluded to, he wrote back: "the *Ponera* was taken by me in Headley Lane in moss under the bushes on the left-hand side of the road, about half a mile from the corner turning away from Mickleham. I dare say it is there still."

His valuable collection of Coleoptera, other than British, was acquired some years ago by the British Museum of Natural History, his library by the Cawthorn Institute, Nelson, New Zealand, in the fauna of which country he had taken so much interest, and where he felt that it would be of more use than in this country.

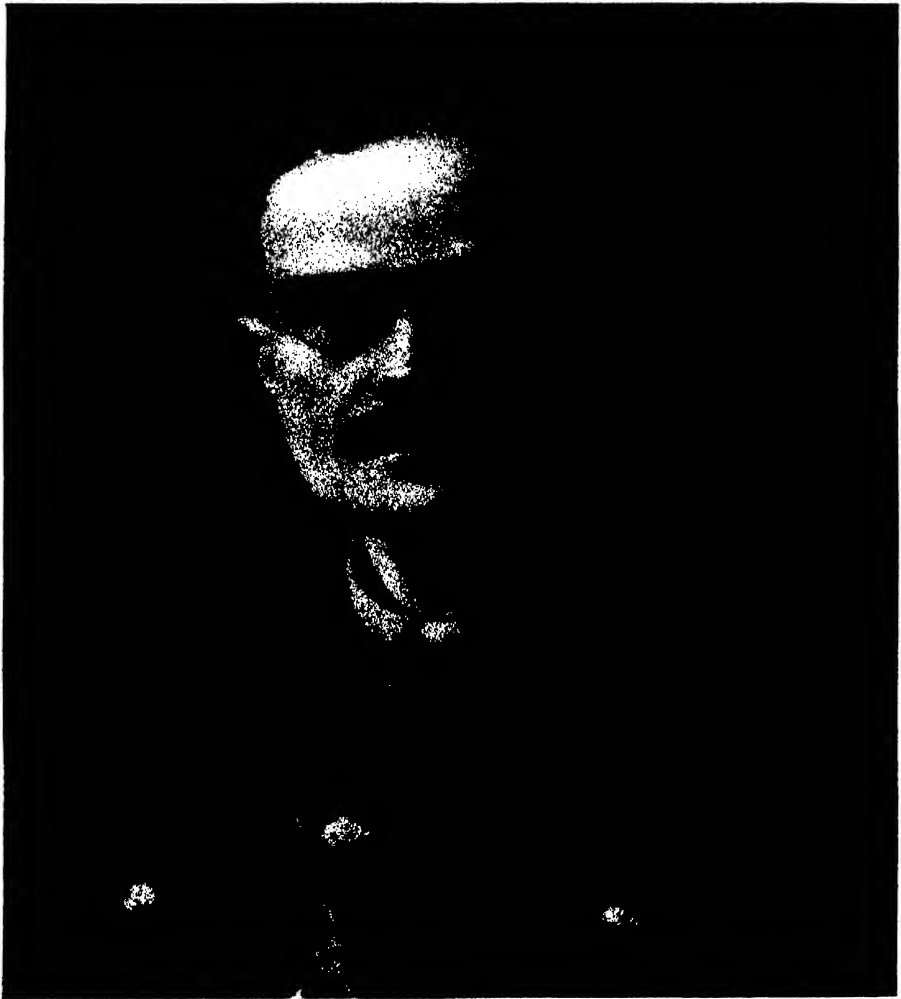
Sharp's work was justly held in the highest esteem by entomologists throughout the world, and its extent was such that those of all countries were concerned. He was an honorary member of the entomological societies of France, Germany, Russia, the Netherlands, Washington, Hawaii, and of the New Zealand Institute. At the time of his death he was the oldest surviving Fellow of the Entomological Society, having joined in 1862, and he acted as Secretary as long ago as 1867. He was President in 1887-88, and Vice-President on several occasions. He was also a Fellow of the Zoological Society and until recent years of the Linnean and an honorary M.A. of Cambridge. He was elected F.R.S. in 1890.

He married in 1875. His wife, a son, and five daughters survive him. Another son, who joined the army in Australia, where he was resident, died in England during the war. His daughter Anne, who is an accomplished entomologist and latterly assisted her father with his work, is the wife of Mr. F. Muir, above referred to as the joint author of one of Sharp's most important works.

We have consulted and are indebted to the Obituary Notices by Commander J. J. Walker ('Entomologists' Monthly Magazine,' October, 1922), Mr. W. J. Lucas ('The Entomologist,' October, 1922), and Mr. F. Muir ('The Entomologist's Record,' vol. 34, p. 186).

R. C. L. P.





*John H. Reilly*

### WILLIAM HALSE RIVERS RIVERS, 1864-1922.

WILLIAM HALSE RIVERS RIVERS died in the plenitude of his powers, after a few hours' illness, on June 4, 1922, of acute intestinal obstruction. No mere recital of the events of his life, or of the honours he received from learned societies, can give any idea of his scientific achievements, and no single individual is capable of doing justice to his varied activities. For the range of his researches included the normal and morbid functions of the nervous system, psychology in the widest sense of the term, and ethnology both practical and theoretical. All these great interests of his life he came to look upon as aspects of the same problem, the biological reaction of man to his environment.

He was born on March 12, 1864, the elder son of the Rev. H. F. Rivers for many years Vicar of St. Faith's, Maidstone. Many of his father's family had been officers in the Navy, and, on his mother's side, an uncle, Dr. James Hunt, was founder and first President of the Anthropological Society. From Tonbridge School he hoped in due course to pass on to Cambridge, but a severe attack of typhoid fever necessitated a year's rest, and in 1882 he entered at St. Bartholomew's Hospital. So great was his intensity of purpose that four years later he passed the M.B. examination of the University of London. On gaining this qualification, he travelled as a ship's doctor to the East, occupying his spare time in a systematic study of Herbert Spencer's philosophy. This was the first of his many journeys, for, as he confessed in later life, desire for change and novelty was one of his strongest mental characteristics.

On his return he became Medical Officer to the Chichester Hospital, and thence in 1889 was unexpectedly summoned to become House Physician at St. Bartholomew's under Dr. Gee. During a happy and fruitful year his mind became diverted from general medicine in the direction of nervous diseases, and in March, 1890, he passed on to a resident post in the National Hospital, Queen Square. Here he came into contact with all that was most vigorous in the neurology of the day. Hughlings Jackson, then in his fifty-sixth year, was an acknowledged master, and, among the younger men, Horsley was pre-eminent for his brilliant enthusiasm and energy. In this atmosphere Rivers not only became a clinical observer of the first order, but found time to develop his leaning towards the more theoretical aspects of neural activity. Here we first met in 1891. For some years I had worked with Ewald Hering in Prague, and Rivers absorbed with avidity the views on colour vision and the nature of vital processes in living matter expressed by this physiologist of genius.

He resigned his post to spend the summer of 1892 in Jena, visiting the hospital and attending lectures on a wide range of subjects, including philosophy, psychology and mental disease. This led to a resolution to

devote his life to psychology, and especially to its morbid manifestations. To this end he became Clinical Assistant at Bethlem Hospital, and began to lecture on experimental psychology at University College.

As a teacher and practitioner in the higher branches of mental medicine, his course seemed clear and his future was assured. Yet, when in 1893 he was invited to Cambridge to lecture on the Physiology of the Special Senses and to found a school of experimental psychology, a post which scarcely offered a living wage, he threw up all his prospects for the opportunity of a wider field of scientific work. With his usual thoroughness, he prepared himself for this work by another visit to Germany, where he carried out a research on the influence of fatigue with Kraepelin in Heidelberg. In October of the same year he took up his residence at St. John's College, and started the first course of practical psychology in this country.

His attention was mainly occupied with problems of vision, and the chapter in Schäfer's 'Text-book of Physiology,' written at this time, remains one of the best general statements on the subject in English, particularly valuable for its clear presentation of the merits and defects of the various colour-theories.

In 1898 began a new epoch in his life; as University lecturer on experimental psychology he was persuaded to accompany the Cambridge Anthropological Expedition to the Torres Straits, to investigate the visual acuity and colour sense of the natives of these islands. He showed that colour-blindness did not exist or was very rare, but that the colour vision of the Papuans was not of the same type as that of Europeans; they possessed no word for blue, and an intelligent native found nothing unnatural in applying the same name to the brilliant blue of sea or sky and to the deepest black. Moreover, he was able to explode the old fallacy that the "noble savage" was endowed with powers of vision far exceeding that of civilised natives. Errors of refraction are, it is true, less common, especially myopia. But, although the feats of the Torres Straits islanders equalled those reported by travellers from other parts of the world, they were due to the power of attending to minute details in familiar and strictly limited surroundings, and not to supernormal visual acuity.

In the course of these observations he collected many family histories and constructed genealogical tables, especially of the small closed community inhabiting Murray Island. It was at once evident that the names applied to the various forms of blood relationship did not correspond to those used by Europeans, but belonged to what is known as a "classificatory system"; a man's "brothers" or "sisters" might include individuals that we should call cousins and the key to this nomenclature is to be found in forms of social organisation especially in varieties of the institution of marriage. A certain term of relationship implies definite duties, privileges and mutual restrictions in conduct. All these facts were clearly demonstrable by the genealogical method, a triumphant generalisation which has revolutionised ethnology. In

1902, the year in which he was elected to a Fellowship at St. John's College, he spent many months amongst the Todas, a polyandrous hill-folk of Southern India. Here he proved his ability as a field anthropologist and established the validity of his previous conceptions of social organisation.

In spite of these preoccupations his interest in the problems of sensation remained undiminished. After division of the radial and external cutaneous nerves in my arm, Rivers investigated the changes in sensibility with all those infinite precautions so necessary because the subject was at the same time an interested observer. For five happy years we worked together on week-ends and holidays in the quiet atmosphere of his rooms at St. John's College.

During these experiments he became imbued with the idea that the functions of the nervous system, at any rate on the afferent side, are the result of integration. A more discriminative mode of response has been superposed on one of a primitive character, which, under normal conditions, is in part inhibited and in part utilised in the final sensation. The excessive reaction to certain stimuli, apparent during the recovery of sensibility, is due to the escape of "protopathic" impulses from the control normally exercised by the "epicritic" system. This conception had a profound influence on Rivers' psychological views and subsequently formed the basis of "Instinct and the Unconscious," the most far-reaching and popular of his writings.

During this period he also undertook a series of investigations on the influence of alcohol and other drugs on fatigue; these were carried out with his usual ingenuity and the most careful experimental controls. By these precise methods he failed to confirm the work of previous observers and concluded that the most important action of alcohol was to lessen control exercised by the higher centres and thus to diminish mental efficiency. Although, under certain conditions, it might act favourably on muscular work, it did so by increasing pleasure and diminishing sensations of fatigue.

The year 1908 saw him again in Melanesia and the results of this expedition are embodied in the "History of Melanesian Society" (1914); this he considered his most original and complete piece of work. He showed that amongst a simple and primitive people social customs and beliefs fall into groups, which can only be explained by supposing distinct waves of immigration. Each fresh infusion led to changes in social organisation still visible in the life of the people long after all other evidence of this admixture has vanished. Similar customs amongst races of different physical characters are frequently due to the direct influence of some immigrant race, and are not of necessity the product of psychical activities common to all humanity. Throughout Melanesian society there is a dual organisation, still betrayed by diverse methods of burying the dead, the drinking of kava or chewing of betel, and the existence of secret societies.

He became increasingly immersed in problems of ethnology, and the outbreak of war found him in Melanesia for the second time. On his return to England he threw himself into practical medicine, becoming one of that brilliant band of workers who made Maghull a centre for the study of



abnormal psychology. In 1916 he was transferred, with the rank of Captain, to the Craiglockhart Hospital for Officers. Here his sound training in clinical neurology stood him in good stead, and he showed his remarkable power of gaining the confidence of young men. In earlier days at Cambridge his general health had been poor, and, in order to accomplish the large amount of work completed during this period, he was compelled to live somewhat the life of a recluse. But his vivid interest in the personality of each individual under his care, and his determination to help, developed a latent capacity to influence deeply all with whom he was brought into contact. At the end of 1917 he became Consulting Psychologist to the Royal Air Force, and acquired a remarkable knowledge of flying, which resulted in a study of the mental qualities required for military aviation.

These varied experiences led him to consider functional nervous disorders from a general biological standpoint. He attempted to bring the abnormal phenomena of mental life into harmony with processes familiar on the physiological level. He conceived of instincts as suppressed forms of primitive behaviour, which may be used in part during normal acts of consciousness or held in check completely. This he thought was analogous to the control exercised by "epicritic" impulses over more primitive "protopathic" reactions. Experience of the War neuroses led him to believe that, when a man regressed to a more instinctive form of conduct under the influence of mental states, his actions assumed an infantile character; this was evident in the content and structure of dreams. "Instinct and the Unconscious," the book in which he put forward these wide generalisations, had a profound influence not only on clinicians, but also on general readers, and his weekly lectures on subjects of psychological interest were attended by students in every branch of thought.

With his return to Cambridge in 1919 began the happiest and most brilliant stage of his career. As Praelector in Natural Science at St. John's College, no formal teaching was required; his enthusiasm, personal influence, and individual knowledge of the undergraduates profoundly stirred the scientific life, not only of the College, but in the University as a whole. The standard he demanded for himself made it imperative for others to keep up to a high level. After the War he felt that he could not return to his life of scientific detachment, and, a few months before his death, he accepted the invitation of the Labour Party to become their Parliamentary candidate for the University of London. He held informal meetings in his rooms on Sunday evenings for discussion of the most diverse subjects; here he was seen at his best.

He was intensely occupied with the drama of human life, and this made him an interested auditor and a valuable counsellor in serious trouble. Triviality he abhorred. Pretentious pomposity withered in his presence, and the incompetent, however high in official rank, trembled before him. He created an atmosphere in which the insignificant could not survive. He was endlessly patient with an honest expression of personal opinion and his charity was boundless; but unsuspecting persons who expressed some

mischievous view were often startled by the vehemence of the reaction they evoked from this modest man of science. With younger men he was extraordinarily gentle, and I have often watched him wait his opportunity to drop into a discussion the exact word which brought together two disputants who had failed to understand one another. With him all conversation was constructive; he disliked barren argument or talking for effect and mastery.

He was an unusually trustworthy critic of the work of others; his opinion was sought by the most diverse persons and administrative bodies, and his verdict was given without hesitation. When he praised he carried immediate conviction, and his rare interventions in the debates of a committee of which he might happen to be a member were always effective.

His strongest characteristic was his intellectual rectitude, for he was never afraid to face the consequences of any view he might hold, and was always prepared to yield to cogent contradictory reasons. Whatever course of action he undertook was approached with the same quiet intensity; however adventurous might be his opinions, he wasted no time in tilting at windmills. The loss to science and to the University caused by his death is irreparable; for he aroused in those who came into contact with him a passionate consciousness of the significance of life and the beauty of organised knowledge.

(A complete bibliography of his writings will be found in "*Man*," July, 1922, vol. 22, No. 61. "*Conflict and Dream*" and "*Psychology and Politics*" appeared after his death, the latter containing an appreciation by Dr. C. S. Myers.)

H. H.

# HENRY JOHN ELWES, 1846-1922.

HENRY JOHN ELWES was born on May 16, 1846, heir to landed property and great wealth. Endowed with unusual powers of observation and organisation, he accomplished much in an active life of over seventy-six years. In this he was aided by his splendid physique and great energy of mind and body. He was pre-eminently a naturalist, imbued with a love and knowledge of plants and animals. He trained himself in scientific work and became an authority on lepidoptera and on trees. He also achieved fame as a traveller, horticulturist, and ornithologist. His life was full of variety and public service.

Elwes was educated at Eton, and, after leaving school in 1862, acquired a knowledge of French at Brussels and of German at Dresden. Few of his school-fellows are now living. One writes:—"Elwes was far fonder of watching the habits of birds in the woods and fields around Eton than he was of games. He was also fond of bird-stuffing, in which he gave lessons to me and two or three other boys. As far as I know, wild animals and birds were then his chief interests in life. I do not think that his love of flowers and of trees, which subsequently produced such fine fruits in literature, had then made its appearance."

On his return from abroad, Elwes served five years in the Scots Guards, retiring, with the rank of captain, in 1870. During this period he began a series of travels, which have not been exceeded in number, duration, or extent by any naturalist. He visited, at one time or another, every country in Europe. He also travelled, for the purposes of collecting and of study, in Algeria, in Asia Minor, in Mexico, and in Chile, once; in India, five times; in China, Japan, and Formosa, twice; in Russia and Siberia, three times; and in the United States and Canada, four times. These expeditions, conducted with great energy, have increased our knowledge of natural history in the branches of ornithology, entomology, and botany, and have contributed to the advancement of gardening and forestry in this country. Elwes also opened up personal relations with scientific men of other nations, and gained friends for us in many lands.

In his travels he often combined sport with natural history. His main amusement was big-game shooting. He repeatedly hunted elk in Norway and chamois and red-deer in Austria; and for nine years was member of a wild-boar shooting syndicate in the Ardennes. His last published article, which appeared in 'The Scotsman,' May 27 and June 3, 1922, was a critical review of the Departmental Committee's Report on the Deer Forests of Scotland, and gave the results of his own experience over a long period. His interest in every pursuit which he took up remained keen to the last.

At the outset of his career as a naturalist, Elwes devoted himself to ornithology, and his discoveries in this subject were undoubtedly important.

He joined the British Ornithologists' Union in 1866, and was its oldest surviving member when elected president in 1921. He contributed numerous articles to 'The Ibis' concerning the birds which he observed on his travels. His first paper (1869) dealt with wild fowl in the Outer Hebrides, and was the result of early trips to these islands and the Orkneys. His first journey abroad as an ornithologist was in 1869, when he spent some time in Greece, Macedonia, the marshes at the mouth of the Danube, and the Crimea, shooting and exploring. He wrote about the birds of Travancore, where he hunted elephant in 1870. Later he contributed field notes concerning the birds of Denmark and of the Altai Mountains. He published in 1872 a monograph of the genus *Henicurus*, and described in 1881 a new *Crossoptilon* from Tibet.

The perusal of the 'Himalayan Journals,' one of the most fascinating books of travel, induced Elwes to visit India, for the first time, in 1870. He accompanied the distinguished naturalist, W. T. Blanford, in an attempt to follow the footsteps of Sir J. D. Hooker through Sikkim. As is well known, one of Hooker's exploits was the unauthorised incursion which he made, in 1849, into Tibet, by a route closed to Europeans since 1783, when Turner visited Lhasa as an emissary of Warren Hastings. It is curious that Elwes repeated this adventure in almost the same dramatic circumstances as Hooker. His companion, Blanford, was not allowed by the Tibetan frontier guards to cross the Donkia Pass, the gate leading from Sikkim into the Tibetan province of Chumbi. Elwes, however, found an unguarded pass close-by, and descended into Tibet as far as the Cholamo Lake, so well depicted by Hooker, and came back, to the astonishment of the Tibetan guards, from the north. Hooker, in a letter to Darwin, mentions the visit to Kew, in 1871, of "Mr. Elwes, a guardsman, who had been up to my most distant passes in the Himalayas, the first to do it since 1849." Mr. J. A. Gammie, who lived for thirty-two years in Darjiling, at various times, entertained Elwes and supplied him with Lepcha porters for his expeditions. He writes:—"The Lepchas are born naturalists, who know by distinctive names nearly all plants, beasts, and birds, and the more conspicuous insects. Elwes, by his personal magnetism and knowledge of Hindustani, in which he talked with them, gained their confidence to a remarkable degree, and they always remembered him as the 'great captain sahib.'"

During the journey through Sikkim, in 1870, Elwes was mainly interested in birds, and his name is commemorated in two species, which he discovered, one in the lower valleys, known as "Elwes's crane," *Porzana bicolor*, and the other, "Elwes's horned lark," *Otocorys Elwesii*, found close to Kongra Lama Pass, at nearly 16,000 feet elevation.

Elwes made a highly interesting contribution to science in his paper, "The Geographical Distribution of Asiatic Birds," which appeared in 1873 as a result of the Sikkim Expedition. He pointed out that the birds of the Himalayas extended to north-west China, and he established the immense Himalayo-Chinese sub-region as a unit. Sir W. T. Thiselton-Dyer regards this paper as an important landmark in the history of distribution. Shortly

after its publication, the collections of Przewalski confirmed the sub-region for the flora. The Himalayan flora is now looked upon as simply the western extension of the Chinese flora, the richest and most varied region in the whole world. To this paper Elwes attributed his subsequent Fellowship of the Royal Society in 1897.

The lavish display of butterflies in Sikkim awakened in Elwes an interest in entomology, and he is said to have regretted, on returning from his first expedition, that he had not paid attention to a group which varied so much with environment. He atoned for this by the extensive collections which he amassed in his subsequent visits to the Himalayas in 1875, 1880, and 1886. His "Catalogue of the Lepidoptera of Sikkim," an annotated list of 530 species, appeared in 1888. He wrote on the butterflies of the Eastern Alps (1879), on those of Amurland, North China, and Japan (1881), and on the genus *Parnassius* (1886). He travelled far and wide in search of material, making numerous trips to the Alps and Pyrenees, and long journeys to the United States, Mexico, and Canada in 1885, 1888, 1893, and 1895. He was president of the Entomological Society in 1893 and 1894, and in the latter year delivered an address on the geographical distribution of butterflies. His journey to the Altai Mountains in 1898 was most successful in obtaining new species.

The 'Transactions of the Entomological Society' for 1889 and 1903 contain vivid accounts of his travels in the Altai Mountains and in the Andes of Chile. The last of his twenty-eight entomological papers appeared in 1906, and describes the rich collections made by the officers of the Tibet Frontier Commission, 1903-1904. His systematic work is acknowledged to be very sound. Fifteen species of butterflies commemorate his name. By 1900 he had amassed the most complete collection known of the butterflies of the North Temperate Zone. He gave 15,000 of his best specimens to the Natural History Museum, South Kensington; and between the years 1900 and 1905 he re-arranged the whole of their Palearctic series of butterflies, a task requiring much time and patience. It is doubtful whether any other man had a wider intimate knowledge of the Lepidoptera of the temperate regions of the world.

Elwes became interested in gardening after his marriage in 1871, when he settled at Miserden and began, without a trained man, to learn how to grow common plants. He received instruction at this stage from an eccentric old Quaker, who lived at Painswick in a cottage, with a small garden and a tiny greenhouse, in which rare plants were successfully cultivated. It was only when he succeeded to Colesborne, after his father's death in 1891, that Elwes for the first time employed a professional gardener. He ultimately became a distinguished horticulturist, and it is remarkable how much he achieved in the culture of plants by simple methods. He had the intuition as to the needs of a plant in soil, position, and suitable amount of moisture which makes a real gardener. A friend writes of him: "In his own garden his generosity

was overwhelming. He more than once divided his only specimen of some rare plant and gave me the larger half in spite of all protest."

The garden at Colesborne, in spite of its rather trying soil and climate, became noted for its fine collections of bulbs and succulents, and was enriched with many new and rare plants, which Elwes gathered on his expeditions. His earliest treasures were brought from Asia Minor, where he discovered, in 1874, six new species of *Crocus* and the charming snowdrop, *Galanthus Elwesii*. Other hardy novelties of great beauty were added later from the same region and from the Himalayas. Amongst these may be mentioned three species of *Fritillaria*, *Tulipa undulatifolia*, *Iris cretensis*, *Aster diplostephioides*, and *Allium macranthum*. Nearly every country which he visited yielded new and precious plants, such as *Cypripedium guttatum* from the pine forests of the Altai Mountains, *Cypripedium montanum* from Oregon, *Tricyrtis stolonifera* from Formosa, and *Tulipa primulina*, which he found in the Aures range in Algeria. In 1890 Max Leichtlin gave him a fine collection of South African *Nerines*, which Elwes grew to perfection and did much to improve by hybridisation.

From the time that he took first to gardening till the end of his days Elwes was keenly interested in the 'Botanical Magazine.' No less than 100 of his plants are figured in it, the greatest number that ever came from a private garden. His successful efforts to continue this old-established periodical, when it was threatened with extinction, redound to his credit. It was due largely to his generosity that a sufficient sum was raised to buy the copyright and to make a handsome contribution towards the cost of the purchase of the old stock by the Royal Horticultural Society. Elwes was regular in his attendance at the scientific meetings of this society, and contributed £1000 towards the building of the Horticultural Hall, in Vincent Square. He was selected as one of the Victorian medallists, honoured by the Society for their services to horticulture, at the jubilee of Queen Victoria in 1897.

Elwes made large botanical collections on many of his expeditions, as in Chile, the Altai Mountains, Japan and Formosa. He was attracted to botany through horticulture and arboriculture, but never acquired a technical knowledge of the subject. He brought out the splendid "Monograph of the Genus *Lilium*" in 1880, but the purely botanical part of this work was done by J. G. Baker. Dame Alice Godman, in collaboration with Mr. A. Grove, is producing a supplement to the monograph, which will be in the nature of a memorial to Elwes, who earnestly desired its publication during the last years of his life.

Though often absent from home for long periods, Elwes carried out his duties as a landowner at Colesborne, and took an active interest in agriculture. He was a keen judge of stock, generally attending markets himself, and doing his own buying and selling. He was interested in sheep-breeding, and made some novel experiments, which are recorded in a pamphlet, "Primitive Breeds of Sheep and their Crosses," that he wrote in 1913. He crossed ordinary improved breeds with semi-wild sheep from Soay Isle in the

Hebrides, and with Shetland sheep, etc. The vigorous progeny which resulted proved capable of enduring great hardships of wet and cold, and thrived on the poorest pasture. Their economic value for producing fat lambs, small mutton, and very fine wool, promised to be considerable, but was not tested on a commercial scale. Prof. Barker, of Leeds, was struck with the possibilities of wool-growing disclosed by these experiments, and believes that valuable types could be evolved in the way pioneered by Elwes.

The love of trees is usually late in developing, and Elwes appears to have taken little interest in them until 1888, when he travelled with Mrs. Elwes and Mr. F. Godman through the great forests of Southern Mexico, California and Wyoming. Even then he collected only butterflies and flowering plants. The turning point was in 1900, when he rushed off to the Drina Valley in Bosnia, with the express object of seeing the remarkable spruce *Picea omorica*, which occurs nowhere else in the living state. In the winter of 1901-1902 he visited Southern Chile, "in the hope of introducing new trees and plants to our gardens," and paid particular attention to *Araucaria imbricata* and the southern beeches, which are conspicuous elements of the forest flora in this region. He introduced two of the beeches by seed, and one, *Nothofagus obliqua*, promises to be a tree of great value in England, growing rapidly and producing excellent timber. His later introduction of the Western Larch from Idaho in 1903 was a praiseworthy but unsuccessful effort to find a good substitute for the European Larch, which for many years has been ravaged by disease in our plantations.

His greatest achievement in arboriculture was the publication of 'The Trees of Great Britain and Ireland,' in collaboration with Augustine Henry. This immense work, 2022 pages and 412 plates, appeared in seven volumes and an index part, 1906 to 1913, but it required more than ten years of strenuous labour from the two authors. Between them they visited all the important collections of trees in the British Isles, and they travelled in the native regions of all the introduced species except New Zealand and Australia. No expense was spared in the production of the book, and no pains were omitted in collecting and verifying the information. The purely botanical part was entirely done by A. Henry, but the pages dealing with distribution, history, and cultivation were divided in varying proportion between the two authors, the share of each being shown by his initials. It is for others to appreciate the worth of these volumes, but they certainly gave an impetus to arboriculture and forestry in this country. New ground was broken in the elucidation of the history of a number of important trees in cultivation, the origin of which had been quite unknown. The earliest clue in one group, trees of hybrid origin, was due to Elwes's persistent curiosity in regard to the remarkable oak, *Quercus Lucombeana*, which first came into being in a nursery at Exeter in 1765.

Forestry has come to the front during the last twenty years as a subject of national importance, and in this movement Elwes took a share. His services to British forestry were, indeed, considerable. He joined the Royal English

Arboricultural Society in 1900, and, during his Presidency in 1907, founded the 'Quarterly Journal of Forestry.' To this he contributed from time to time stimulating articles on silvicultural practice and vivid descriptions of some of the foreign forests (Formosa, Slavonia, Portugal) which he visited in search of information. Elwes was also chief founder of the Tubney Arboretum, near Oxford, where groups of many different species are grown under natural woodland conditions. He made a similar plantation at Colesborne, mainly from seed gathered in 1900, and the records of this have been carefully kept. The usefulness of these experimental plots will be recognised in after years.

The better utilisation of home-grown timber was a subject which appealed greatly to Elwes's practical mind. He was convinced that the products of our own woodlands were not sufficiently known or advertised, and that in consequence good material was either wasted or put to unworthy uses. By his writings and public lectures, he spread the knowledge of the good qualities of British timbers amongst architects, furniture-makers and the general public. His own house was decorated with panelling, tables, and book-cases made out of native woods. He paid particular attention to the burrs on the trunks of old trees, which, when cut into veneers, often show curiously veined figure, and are precious in cabinet-making. He was one of the few who recognised the economic value of the many beautiful woods of India and Burma, which were neglected in commerce till recently. During the War he experimented in charcoal-burning, and acted as Chairman of the Sub-Committee on Aeroplane Timbers.

It was partly the love of timbers that induced Elwes to aid forestry education at Cambridge, where the beautiful building of the Forestry School, with its unique collection of foreign and native woods, owes much to his munificence. He was also interested in the teaching of silviculture at the University to the future owners of woodlands.

His public services were many, and some of these have been mentioned above. He was the official representative of Great Britain at the Botanical and Horticultural Congresses held at Amsterdam in 1877 and at St. Petersburg in 1884, and he acted as British Juror in Forestry and Horticulture at the St. Louis Exhibition of 1904. He was appointed Scientific Member of the Macaulay Mission to Tibet in 1886, which was not proceeded with owing to the jealous attitude of the Chinese Government. Elwes made a journey to the Khasia Hills instead. His last voyage to India was in 1914, when he entered by special invitation the almost forbidden land of Nepal. His account of its people and government in 'Journ. R. Society of Arts,' February 12, 1915, is full of sympathy and understanding.

Elwes kept up his activities and interests until the last year of his life, when he was attacked by a fatal illness. He passed away on November 26, 1922.

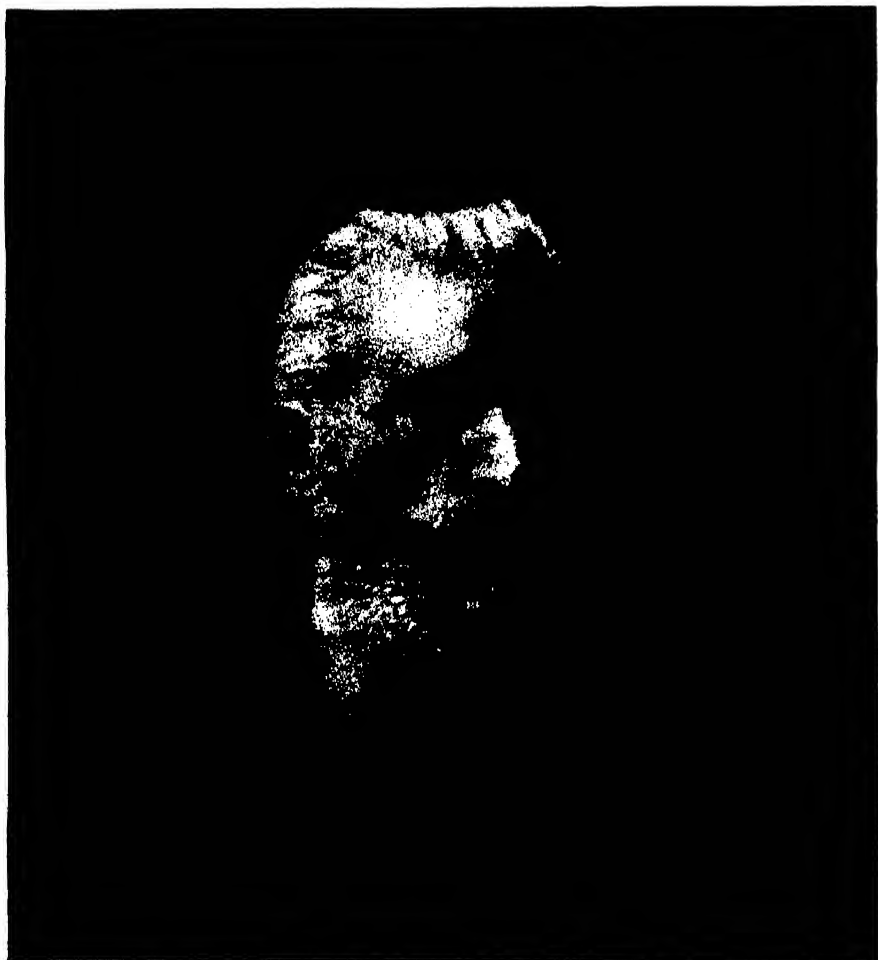


### CHARLES IMMANUEL FORSYTH MAJOR, 1844-1923.

DR. CHARLES IMMANUEL FORSYTH MAJOR, who died in Munich on March 25th, 1923, was the son of Charles Forsyth Major, a Scottish Divine. He was born in Glasgow on August 15th, 1844; when only a few weeks old he was taken to Constantinople and spent most of his life abroad. He was educated in the universities of Basel, Göttingen, and Zurich, graduating in Medicine at Basel in 1868. While at this university he came under the influence of the well-known palæontologist Prof. Rüttimeyer, for whom he had the greatest admiration and whose methods he followed in his own palæontological researches. After graduation he practised medicine in Florence, but already devoted much time to the study of fossil mammals, working for a while under Prof. Meneghini. His first paper was entitled "Note sur les Singes fossiles trouvés en Italie," published in Milan in 1872. This was followed by a long series of papers on fossil mammals from the caverns and Pliocene deposits of Italy: these were published in French, German and Italian. During this period he also finished two important memoirs on the dentition of the Eocene rodents of Switzerland and South Germany ('Palæontographica,' vol. XXII, 1873), and on the early horses ('Abhandl. Schweiz. Pal. Gesellsch.,' 1877-80). In 1877 he was sent by the Italian Government to Calabria and afterwards to Corsica, Sardinia and Sicily, for the purpose of collecting fossil vertebrates.

In 1886 he finally gave up the practice of medicine and from that year till 1888 he was investigating the recent fauna and flora of the islands of the Grecian Archipelago, also collecting fossil vertebrates and paying special attention to Carpathos and Samos. At Mytilini in the latter island he discovered a rich mammalian fauna of Upper Miocene age, resembling that of Pikermi near Athens, but including many other remarkable animals, such as *Samotherium*, *Prostrepsiceros*, *Criotherium*, a species of *Orycteropus*, and an ostrich. In these expeditions he was assisted by his friend M. Barbey, who presented a large part of the collection of fossil vertebrates to the Collège Galliard at Lausanne. The remainder was purchased by the British Museum. He wrote some preliminary notes and lists of his discoveries, but the great memoir he intended to publish never appeared.

About this time he also made a large collection of Pliocene mammals from Monte Olivola, in the Carrara mountains: these remains, which have never been fully described, are also in the British Museum. In 1893 Dr. Major came to London, where he worked in the Natural History Museum. Soon after his arrival there the skull of a giant Lemur from Madagascar was discovered; this he described in the "Philosophical Transactions" under the name



George Washington



*Charles Immanuel Forsyth Major.*

*Megaladapis madagascariensis*. In consequence of this discovery he went to Madagascar with the assistance of the Royal Society and spent about two years there, making large collections of both the recent and fossil mammals and birds. The fossil mammals he described in the 'Philosophical Transactions,' and elsewhere.

In 1899 there appeared one of his most important memoirs entitled "On the Fossil and Recent Lagomorpha," dealing exhaustively with the history of the dentition in this group and also with the structure of their limbs. This paper was published in the 'Transactions of the Linnean Society.' About 1900 Dr. Major became interested in the Okapi, and a great part of the osteological material available for the study of that interesting animal was placed in his hands; he made an elaborate study of its structure and affinities, but only some brief notes ever appeared. In 1906 Dr. Major was granted a Civil List pension; he continued for some time to contribute papers to various periodicals and was engaged in preparing a Catalogue of the fossil Rodentia in the British Museum. About the end of 1908 he went to Bastia in Corsica, where he spent most of the remainder of his life. During these later years he made collections from the caverns of Corsica and was engaged in preparing an account of his discoveries at the time of his death.

Only those who were personally acquainted with Dr. Major can appreciate the immense amount of work he did or the profundity of his knowledge of the fossil mammals; the writer of this notice and many others will always be indebted to him for help and advice willingly given.

Dr. Major was a remarkable linguist and wrote several papers on philological subjects, mostly dealing with dialect names of animals. He was elected a Fellow of the Royal Society in 1908, and was also a member of several foreign societies.

C. W. A.



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